

Fishery Bulletin

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July 1971

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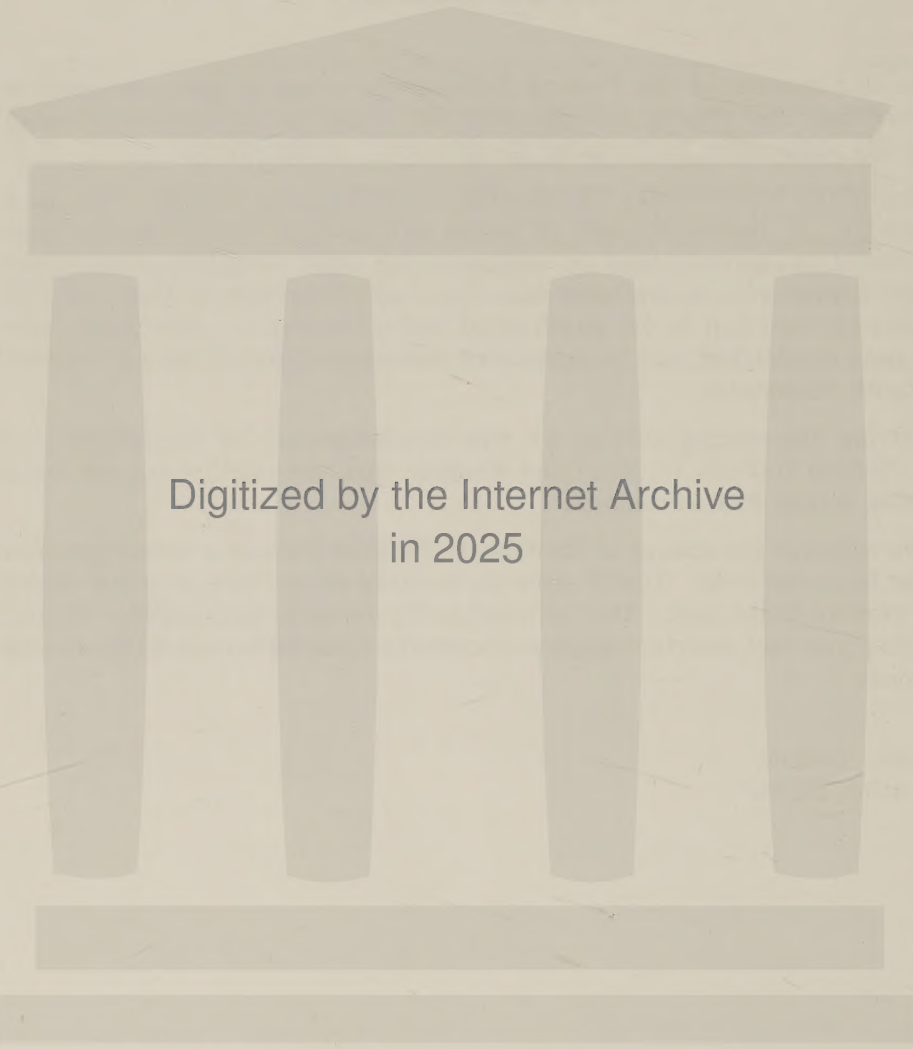
As this number of the *Fishery Bulletin* was going to press, we were informed that the Office of Management and Budget has approved our request that the publication be formally recognized as a periodical.

Beginning with Volume 70, Number 1, the *Fishery Bulletin* will appear quarterly. A limited number of copies will be distributed free of charge, primarily to libraries and in exchange for other scientific publications. Other copies will be available from the Superintendent of Documents by annual subscription or by purchase of individual copies. The prices have not been established, but we have been encouraged to believe that they will be quite reasonable.

During the coming months we will circularize each of the recipients of the *Fishery Bulletin* to determine whether they are eligible to continue to receive copies free of charge.

We welcome the change of the *Fishery Bulletin* from an irregularly issued serial to a quarterly. It will allow us to carry about twice as many papers per year as in the past. Our editorial policy remains unchanged—we hope to bring you each quarter significant scientific contributions in all the marine sciences.

Reuben Lasker,
Scientific Editor



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INTERNAL DEFENSES OF CRUSTACEA: A REVIEW¹

CARL J. SINDERMAN²

ABSTRACT

Studies of the internal defenses of Crustacea have a discontinuous history, which began in the late 1800's. Elaborate early studies of phagocytes and humoral factors in the hemolymph have been extended with renewed vigor during the past decade. As is true for other invertebrates studied, phagocytosis of foreign particles by fixed and mobile cells in the crustaceans is augmented by naturally occurring bactericidins, lysins, and agglutinins. A few instances of experimental enhancement of titers of such humoral factors by previous exposure to foreign protein have been reported. Specificity of natural and experimentally enhanced humoral factors is much lower than that of vertebrate immunoglobulins, but probably such factors act synergistically with cellular protective mechanisms, as they do in the vertebrates.

Phagocytosis by fixed and mobile cells in gills, pericardial sinus, and sinuses at the bases of appendages seems to be a principal defense perimeter in many crustaceans. Efficacy of phagocytes in destroying invading microorganisms varies, depending on the species of the microorganism, as well as host physiology and environmental factors. Phagocytic activity is enhanced by hemolymph factors, which, in addition to immobilizing and agglutinating the invading organisms, also sensitize them to phagocytosis.

Hemolymph factors, most of which seem to be of cellular origin, may also have bactericidal or lytic activity, leading to extracellular destruction of microbial invaders. A few recent studies indicate that effects of hemolymph factors may be enhanced experimentally by injection of killed or living microorganisms.

The number of known microbial diseases in crustaceans is greater than that known in most other invertebrate groups, with the possible exception of the Mollusca and Insecta. The number and depth of studies concerned with internal defense mechanisms of Crustacea are similarly greater than those of most other invertebrate groups—again with the possible exception of the mollusks and insects. One microbial pathogen of Crustacea that has received adequate attention is *Gaffkya homari*—a gram-positive coccus which causes a fatal septicemia in lobsters and is capable of infecting other decapods. An elaborate series of studies in several laboratories has elucidated many details of the host-parasite relationship and has also provided extensive information about the internal defenses of a number of the larger Crustacea. *Gaffkya* constitutes a test microorganism of choice for future studies of disease processes in crustaceans.

Thus the available information about cellular and humoral defenses of Crustacea against invasion by foreign protein constitutes a significant part of what we know about such processes in the invertebrates. Phagocytosis, augmented by humoral factors with low specificity, seems to be the fundamental means of internal protection in the crustaceans and in other groups of invertebrates as well.

Investigations of the internal defense mechanisms of invertebrates against disease have progressed with renewed vigor in the past decade. Literature has accumulated to the point where condensation and summarization of information about certain invertebrate groups, such as the Crustacea, seem justified.

The large number of published reports on internal defenses of Crustacea can provide a good indication of the extent of our knowledge of immunity in marine invertebrates. Beginning with the pioneering work on phagocytosis and the entire process of inflammation by Metchnikoff (1884, 1893, and 1905), and on humoral factors described in the very extensive (but sometimes poorly documented, in terms of procedures and data) work of Cantacuzène (1912-34), the Crustacea have often been animals of choice in studies of internal defense mechanisms. A significant

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body of literature has accumulated, with conspicuous bulges in the early years (1884-1930) and in the past decade (1960-70), but with a very narrow waist during the period 1930-60.

Studies of humoral defenses of the larger Crustacea during the past 5 years (1965-70) are curiously reminiscent of work reported by Cantacuzène and his associates during the period 1912 to 1934, with the very important difference that most of the modern work includes elements that were largely missing from earlier reports, such as details of procedures, supporting data, adequate controls, and attempts at quantitation of results.

Because so much of the literature produced before 1940 lacks adequate quantitation and fails to provide details of techniques used, it is often difficult to relate results to those of more recent studies. Bang (1967b) has deliberately addressed himself to a repetition of earlier studies but has used modern methods in an attempt to improve the relationship. Other recent reports, even though based on species other than those used in earlier studies, constitute reexaminations of the concepts and general findings of the early investigators.

It is difficult to determine why the early work on immune responses in invertebrates so effectively begun by Metchnikoff, Cantacuzène, Cuénot, Bruntz, and others during the late 19th century and the early 20th century seemed to lose impetus and then virtually cease until recently. It is apparent from the literature, though, that research on invertebrate defenses, initiated so auspiciously, receded for a number of decades to the backwaters and eddies of the mainstream of advances in immunology, which was concentrated on the homoiothermic vertebrates. This may be explained in part by a natural and necessary concentration of research interest on human and mammalian immune responses (most immunologists were—and still are—generally associated with medical schools and hospitals). Part of the explanation also may be that earlier work failed to disclose any defense mechanisms in invertebrates that seemed fundamentally or conceptually different from those that were being elucidated for the vertebrates. More importantly, the explanation may have

been that much of the earlier work failed to indicate any immunologic responsiveness in the invertebrates tested. In spite of occasional successes, the “inability of invertebrates to respond to the introduction of antigen by formation of antibodies” became a sort of dogma among many of the early biologists, as was pointed out by Cantacuzène (1923b). Failures to find responses may have been due partly to choice of inoculum with negligible antigenicity in the experimental invertebrate. Two factors may have caused the recent resurgence of interest among biologists in comparative immunology (which is gradually beginning to include the lower vertebrates and the invertebrates): (1) an evident need to reexamine the conceptual and evolutionary basis of immune responses and (2) an interest in understanding the internal defenses of invertebrates, which allow them to survive in a microbe-rich environment even though they lack the specific antibody response characteristic of most vertebrates.

The subject matter of the present review is one that has been treated previously (Cantacuzène, 1923b; Huff, 1940; Baer, 1944; Bang 1967b; Levin, 1967; Tripp, 1969; Rabin, 1970b; Bang, 1970). Many of those papers, however, were broad considerations of the invertebrates as a whole, and discussions of internal defenses of Crustacea were often more or less incidental. It is interesting—as Good and Papermaster (1964) pointed out—that no review of invertebrate immunity has emphasized induced responses. A recent, and excellent, 2-volume text on the physiology of Crustacea (Waterman, 1960) does not include a detailed consideration of the very important subject of internal defenses, except for reference to hemocytes and phagocytosis (Maynard, 1960; Parry, 1960), and a consideration of hemolymph coagulation (Florkin, 1960).

The general plan for this review is to discuss some of the early literature, after a brief preliminary statement about concepts and terminology, and a summary of known diseases of Crustacea. More recent studies will then be considered by categories of cellular and humoral systems: phagocytic, bactericidal, lytic, agglutinating, precipitating, phage clearing, and anti-

toxic. A final section will attempt a detailed review of the demonstrated systems of internal defenses of lobsters and other crustaceans against the microbial pathogen *Gaffkya homari*—which is one of the best examples of a test system for invertebrates for which existing information is adequate.

CONCEPTS AND TERMINOLOGY

Before proceeding with an examination of internal defenses of crustaceans, a brief review of some of the terminology may be relevant. “Resistance” and “susceptibility” have often been used interchangeably and reciprocally, but as Schneider (1951) and Stauber (1961) pointed out, insusceptibility and resistance should probably be considered as separate biological phenomena. Insusceptibility refers to those existing external or internal features of an animal—morphological and physiological—which deny access to a potential pathogen, or prevent its successful establishment and survival. Resistance, on the other hand, has been defined by Read (1958) as “those changes in the physiological state of the host which represent a *response* to present or previous contact with the parasite or with a similar chemical entity.”

Resistance (and its equivalent—“immunity”), defined as host response, can then be considered as “innate” (natural) or “acquired” (induced). Innate resistance includes responses to primary contact with a pathogen, and acquired resistance includes responses that develop after primary contact. The distinction between the two types of response is not, however, always as definitive as might be preferred. Acquired resistance is often characterized by enhanced responsiveness to subsequent contact with a pathogen.

Resistance, whether innate or acquired, may be cellular or humoral. The cellular defense mechanisms are based largely on activities of leucocytes (hemocytes of invertebrates) and include: *phagocytosis*—engulfment and often digestion of foreign particles, *leucocytosis* (*hemocytosis*), and *leucocytic* (*hemocytic*) *infiltration*—the mobilization of hemocytes in the blood stream and their migration to invaded or injured tissues; *coagulation*—the formation of cellular

or extracellular clots to close gaps in the circulatory system and to immobilize microorganisms; and *encapsulation*—the surrounding of large masses of invading material by phagocytes and fibrocytes. Humoral defense mechanisms, which probably depend on cellular secretions or cell disruption, and act synergistically with cellular defenses, include agglutinating, lytic, precipitating, and bactericidal systems, and other activities (Figure 1).

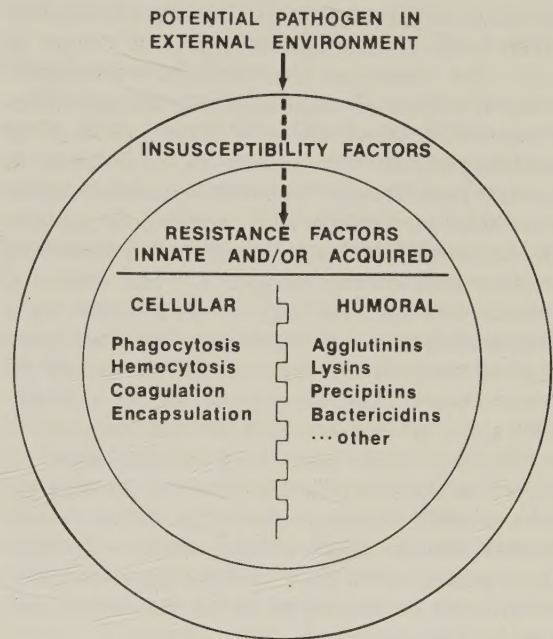


FIGURE 1.—Mechanisms of internal defense.

Antibodies (in the vertebrate sense of specific immunoglobulins) have not been demonstrated in invertebrates, although less specific antibody-like activity is common. Since antibodies have not been demonstrated, it is probably technically incorrect to use the term “antigen” with invertebrates. The semantics involved will be considered in the discussion section, but for convenience the term will be used in this paper. Furthermore, it must be made clear that when lysins, precipitins, agglutinins, etc. of invertebrates are discussed, no attempt is made to

homologize them with vertebrate factors. The terms merely indicate the type of activity produced.

DISEASES OF CRUSTACEA

An impressive array of diseases afflicting the Crustacea has been described (summarized in Sindermann, 1970, and Bang, 1970). A number of these diseases are of microbial etiology, and Koch's postulates have been satisfied for several of them. Some of the published reports concerning crustacean diseases include information about host defenses against infection, others do not. The literature also contains information about a variety of experimentally induced infections in Crustacea, many of them produced by microorganisms not known as pathogens in natural populations. Such experimental studies have been particularly useful in elucidating possible internal defense mechanisms—augmenting studies with known pathogens. The following summary is just that and is not intended as a detailed treatment of crustacean diseases. Some general background information on known diseases seems important, however, to any consideration of internal defense mechanisms.

The only virus disease of invertebrates reported in the scientific literature is one that occurs in crabs, *Portunus depurator*, on the French Mediterranean coast (Vago, 1966). Disease signs mentioned in his very brief paper included progressive darkening of the exoskeleton, paralysis, and death.

Bacterial infections of various Crustacea have been described, beginning with a disease of beach hoppers on the French coast caused by luminescent bacteria (Giard and Billett, 1889). Experimental infections were obtained by injecting cultured microorganisms, and some of the crustacean species tested exhibited varying degrees of resistance to experimental infection. Another luminescent disease was reported in sand fleas (*Talorchestia longicornis* and *Orchestia platina*) from Woods Hole, Mass., by Inman (1927). Luminescent bacilli were cultured, and experimental infections obtained. Luminescent bacteria were also isolated from the digestive tracts of nonluminous sand fleas. A bacterial disease

of *Gammarus marinus* was reported from England by Tait (1917), in which signs of disease included change in color of the infected amphipods from brown to opaque yellowish-white, reduction in numbers of blood cells, and absence of coagulation of hemolymph. Among the larger decapod Crustacea, a severe bacterial disease of lobsters caused by gram-positive cocci, *Gaffkya homari*, was recognized in 1947 (Snieszko and Taylor, 1947) and has been the subject of intensive studies since then (to be considered in detail later in this paper). Experimental infections and resultant mortalities of blue crabs, *Callinectes sapidus*, from Chesapeake Bay were reported by Krantz, Colwell, and Lovelace (1969) with *Vibrio parahemolyticus*. The microorganism has been isolated from mollusks, fishes, and sediments in various parts of the world and is known as a cause of human gastroenteritis in the Orient. Additionally, several examples of "shell disease"—erosion of the exoskeleton by chitin-destroying bacteria—are known in lobsters, crabs, and shrimps (Hess, 1937; Rosen, 1967, 1970; Anderson and Conroy, 1968).

Fungus diseases of Crustacea are surprisingly numerous in reports dating back to Metchnikoff (1884), who described fatal infections of *Daphnia* caused by *Monospora bicuspidata* and who first emphasized the crucial role of phagocytosis in determining the outcome of infection. A yeast infection in sand hoppers, *Talitrus*, from the coast of France, was reported by Herrmann and Canu (1891). Experimental infections from exposure to cultured microorganisms were fatal to *Talitrus* in 20 to 25 days. Phagocytosis was marked in such infections, and the hemolymph became milky in advanced cases. Crabs (*Carcinus maenas*), prawns (*Palaemonetes varians*), and crayfish (*Astacus fluviatilis*) were not susceptible to the experimental infections. Pixell-Goodrich (1928) described another yeast infection which was epizootic in *Gammarus* from a stream in England. The pathogen *Cryptococcus gammari* reproduced in the hemolymph and rendered it milky in color, coagulation was retarded, and heavily infected individuals died. Phagocytosis was active and sometimes successful in overcoming infections. Hypertrophy of fixed phagocytic cells was common.

One of the most severe, widespread, and long-continuing epizootics known in invertebrates has affected and still affects European crayfish. It is caused by the fungus *Aphanomyces astaci* (although other microorganisms have been variously associated with mortalities). Known as "Krebspest," the disease swept through crayfish populations of Europe beginning about the turn of the century (Schikora, 1906, 1926; Schäperclaus, 1935; Nybelin, 1935; Mannsfield, 1942; Unestam, 1965; Gordon, 1966). Apparently resistance differs among species—the American crayfishes, for example, seem less seriously affected by the pathogen in experimental studies.

Other fungus diseases of Crustacea include a systemic infection of pea crabs, *Pinnotheres*, from English sea mussels by *Leptogorgia marina* (Atkins, 1929, 1954a); a systemic disease of cultured prawns, *Palaemon serratus*, in England, caused by *Pythium* sp. (Anderson and Conroy, 1968); a gill infection of pandalid shrimp, *Dichelopandalus leptocerus*, from the western North Atlantic, caused by a chytrid-like microorganism (Uzmann and Haynes, 1969); and gill infections of lobsters, *Homarus vulgaris* and *Palinurus vulgaris*, in Italy, caused by *Ramularia branchialis* and *Didymaria palinuri*—both Fungi Imperfecti (Sordi, 1958).

Fungi also infect and destroy egg masses of Crustacea. Eggs of blue crabs, *Callinectes sapidus*, from Chesapeake Bay may be infected by *Lagenidium callinectes* (Couch, 1942; Newcombe and Rogers, 1947; Rogers-Talbert, 1948); and eggs of pea crabs are often infected by *Plectospora dubia* and *Pythium thalassium* (Atkins, 1954b, 1955).

Among the many protozoan diseases of Crustacea, those caused by microsporidians are probably the most destructive. *Nosema* sp. and *Plistophora cargo* destroy body muscles of blue crabs (Sprague, 1965, 1966); *Nosema pulvis* and *Thelohania maenadis* infect muscles of green crabs, *Carcinus maenas* (Pérez, 1905a, 1905b, 1907). Other microsporidian infections of body muscles in Crustacea include those produced in *Gammarus* by *Theileria* sp. and *Nosema* sp. Necrotic muscle fibers containing microsporidian spores were destroyed by phagocytes (Pixell-

Goodrich, 1928). Other pathological effects of microsporidians on gammarids have been recently reported by Bulnheim (1967) and Bulnheim and Vávra (1968). Crayfish muscles are attacked by Microsporidia of the genera *Thelohania* and *Nosema* (Sprague, 1950b; Pixell-Goodrich, 1956; Sogandares-Bernal, 1962). Microsporidia are also significant pathogens of shrimps. Sprague (1950a), Woodburn et al. (1957), Iversen and Manning (1959), Iversen and Van Meter (1964), and others have described infections of body muscles and gonads of shrimps from the Gulf of Mexico and European waters, caused by a number of representatives of the genera *Thelohania* and *Nosema*.

Other protozoan diseases of crustaceans include those caused by ciliates, an ameba, and gregarines. A ciliate, *Anophrys sarcophaga*, causes a fatal disease in shore crabs, *Carcinus maenas*, of Europe. The disease, and host responses to infection, will be considered in detail in a later section. Other parasitic ciliates occur in the hemolymph of Crustacea. *Paradinium* sp. and *Syndinium* sp. occur in calanoid copepods. *Syndinium* causes gonad destruction, while *Paradinium* colors the host a deep red (Gordon, 1966). *Hematodinium* sp. has also been reported by Gordon in *Carcinus*. A suctorian, *Ephelota gemmipara*, can seriously reduce production of lobster larvae (Dannevig, 1928, 1939). An ameboid parasite, *Paramoeba pernicio*, causes a fatal disease (called "gray crab disease") in blue crabs from the Atlantic coast of the United States (Sprague and Beckett, 1966, 1968; Sprague, Beckett, and Sawyer, 1969; Sawyer, 1969). Hemolymph of infected crabs becomes cloudy and often incoagulable; in some individuals most of the cells in the hemolymph are amebae (Sawyer, Cox, and Higginbottom, 1970). A great number of gregarines occur in Crustacea of all kinds, but their pathogenicity seems slight, except for some destruction of the digestive epithelium resulting from heavy infections (Ball, 1938; Théodoridès, 1961, 1962; Tuzet and Ormières, 1961; Kruse, 1959a, 1959b).

Helminth diseases of crustaceans seem less abundant and less severe in their effects than those of microbial etiology. Trematode metacercariae encyst in muscles and hepatopancreas of

crabs, and larval cestodes, acanthocephalans, nematodes, and leeches occasionally have been reported from crabs, shrimps, and lobsters (Sindermann, 1970).

Crustaceans are frequently parasitized by other crustaceans—sometimes with serious effects on the host. Rhizocephalan barnacles are endoparasites of crabs, causing gonad degeneration and other morphological changes. Epicaridean isopods may produce similar changes in crabs and shrimps. Copepods sometimes parasitize crab eggs, as well as the gills of lobsters (Sindermann, 1970).

INTERNAL DEFENSE SYSTEMS

Studies of crustacean internal defenses published during the last decade have augmented earlier studies and have provided additional data to support generalizations and principles already enunciated, but none has yet provided the factual basis for new or different concepts. Because additional precision in terminology is now available, it is possible to consider the internal defense systems of Crustacea under the following headings: cellular (phagocytic), bactericidal, lytic, agglutinating, precipitating, phage clearance, antitoxic, and others. It should be obvious that these systems are not mutually exclusive and may often interact or even share components to protect the individual animal from invasion by potential pathogens. Largely for ease of description, the systems will be considered consecutively, even though many may act either in concert or simultaneously.

PHAGOCYTOSIS AND OTHER CELLULAR DEFENSES

The earliest study of phagocytosis in Crustacea concerned infections of *Daphnia* by the fungus *Monospora bicuspidata* (Metchnikoff, 1884). The fungus spores in the haemocoel were phagocytized and digested; the rapidity and vigor with which phagocytosis occurred determined the outcome of the infection. If some spores escaped phagocytosis, germinated, and formed conidia, the infection became generalized and the

host died in a few days. If all the fungus spores were phagocytized and destroyed, the infection was arrested. Thus the speed and effectiveness of phagocytic action in some individuals, possibly mediated by humoral factors, determined survival. Absence of phagocytosis inevitably led to death.

Hemocytes of Crustacea were investigated by Cattaneo (1888b), Cuénot (1895, 1897, 1905), and Bruntz (1907). Cattaneo described the amebocytes of *Carcinus maenas*; Cuénot reported blood forming tissues—nodules of lymphoid cells in the blood sinuses—in decapods and described “phagocytic organs” in the hepatopancreas of decapods and amphipods; and Bruntz published an extensive paper on the hemocytes of many of the crustacean groups, distinguishing granular and hyaline hemocytes. Bruntz also described a “phagocytic organ” in gammarids; his 1907 paper reviewed an extensive series of his own studies (15 reports) published during the period 1903-1907 by the Société Biologique de Paris. Other early studies of crustacean hemocytes include those of Hardy (1892), Tait (1918a, 1918b), and Tait and Gunn (1918).

Following the classical early studies of Metchnikoff, Cuénot, Bruntz, and others, which elucidated the critical role of phagocytes in the internal defenses against microorganisms, phagocytic cells have received greatest attention from vertebrate immunologists. General principles that have emerged from the more recent studies of phagocytosis in vertebrates undoubtedly apply as well to invertebrates. Among the papers that have contributed to understanding of phagocytosis are those of Wright and Douglas (1903), Wood, Smith, and Watson (1946), Wood (1953), Robineaux and Frederic (1955), Suter (1956), Rowley (1960), Rogers (1960), Evans and Karnovsky (1961), and Spector and Willoughby (1963). Reviews of phagocytosis have been published by Hirsch (1965) and Aarum (1967).

The mechanism of intracellular degradation of phagocytized microorganisms has been described in general terms for the vertebrates (Figure 2). Lysosomes—granules in the cytoplasm of phagocytes—contain antibacterial substances and hydrolytic enzymes (Cohn, Hirsch, and Wiener, 1963). The lysosome membrane

fuses with the vacuolar membrane within the phagocyte, releasing antimicrobial components into the vacuole (Robineaux and Frederic, 1955; Hirsch, 1965; Aarum, 1967). Evidence for comparable intracellular events in invertebrates is sparse, but Janoff and Hawrylo (1964) reported lysosomal enzymes in clams and starfish, and Eble (1966) found hydrolytic enzymes in oyster phagocytes.

Invading microorganisms are subjected to antimicrobial factors both inside and outside the phagocytes. Substances of presumed cellular origin, such as lysozyme, occur in the phagocytes and the plasma. A great array of such antimicrobial factors was identified in vertebrates (Skarnes and Watson, 1957; Elberg, 1960; Hirsch and Cohn, 1960; Landy, 1960; Coombs, Coombs, and Ingram, 1961; Mackaness, 1962; Miles, 1962), and some counterparts were recognized in invertebrates. McDade and Tripp (1967), for example, reported lysozymes in oyster hemolymph.

In the vertebrates, specific and nonspecific serum proteins increase the speed and effectiveness of phagocytosis—the opsonizing effect (Wright and Douglas, 1903; Suter, 1956; Row-

ley, 1960). Sensitization of bacteria with serum factors is not always a necessary prelude to phagocytosis, however, as was pointed out by Wood, Smith, and Watson (1946) and Wood (1953). In the absence of other host responses, early phagocyte activity may be important in preventing infection.

Phagocytosis, then, constitutes the keystone to resistance. As Aarum (1967) mentioned, "...the organism's ability to oppose infection precisely follows the phagocytes' ability to function optimally. Resistance is lowered by a lowering of phagocytic activity." Phagocytosis can occur at the site of a lesion, in the filtering tissues and organs of the circulatory system, and (to a lesser extent) in the body fluid itself. Groups of fixed phagocytic cells are present in many crustaceans, most commonly in the sinuses and lacunae of the gills and at the bases of the legs.

Phagocytes agglutinate, aggregate, and cooperate in defense—forming nodules (the "nodules leucocytaires" of Cuénot, 1898), which are also known in annelids, mollusks, echinoderms, and other invertebrates. In a number of animals the nodules are brown, due to presence of large numbers of brown granules in the phagocytes, which may be excretion products or decomposition products. In *Gammarus*, the phagocytes composing the nodules secrete a clear yellowish chitinous substance around the parasites. This secretion gradually becomes dark brown. The nodules appear as conspicuous black spots in infected individuals (Pixell-Goodrich, 1928) and are found frequently in gills and appendages. It should be clearly understood, however, that there are few detailed modern studies of phagocytosis in crustaceans or other invertebrates. Data from *in vitro* studies are particularly scarce, so there should be no implication that the kinetics, energetics, or other aspects of phagocytosis are fully understood.

Hemocytes of crustaceans and other invertebrates also act in other ways to protect the individual from overwhelming microbial invasion. Hemocytosis and hemocytic infiltration have been described in a number of invertebrate groups. Manifestations in invertebrates and vertebrates involve proliferation of hemocytes, changes in permeability of blood vessels, leakage

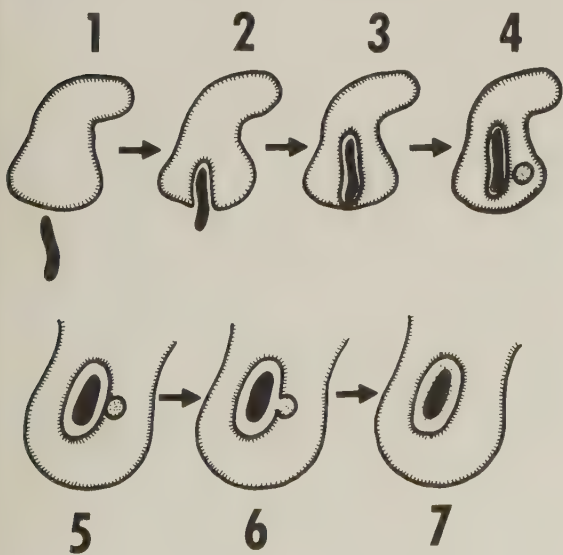


FIGURE 2.—Role of cell membrane and lysosome breakdown in phagocytosis. 1-4; phagocytosis; 5-7: lysosome activities. (Redrawn from Hirsch, 1965.)

of blood fluids into tissues, adherence of hemocytes to blood vessel walls, and migration of hemocytes into tissues around areas of injury or parasitic invasion.

The involvement of hemocytes in coagulation or clot formation is a complex one, inasmuch as either cellular or extracellular clots may be formed. Intravascular cellular clots adhere to walls of blood vessels and spaces, producing stasis, and once they are formed, persist for some time. Extracellular clots, resulting from release of constituents of hemocytes, can inhibit microbial motion and thus render microorganisms more vulnerable to phagocytosis. Since Fredricq (1879) first pointed out that in Crustacea coagulation of hemolymph involves cell agglutination as well as plasma coagulation, others have demonstrated similar characteristics in a number of invertebrate groups. The release of a component from hemocytes and the role of this component in initiating coagulation of plasma were reported by a number of authors beginning with Halliburton (1885). Löwit (1889) observed the rapid disruption of hemocytes and the rapid clotting characteristic of most Crustacea and concluded that a causal relation existed. Hardy (1892), Tait and Gunn (1918), Tyler and Scheer (1945), and George and Nichols (1948) all provided data which supported the conclusion that a component from certain hemocytes acts with fibrinogen of plasma to form fibrin clots.

Bang (1967c, 1968) demonstrated that in the hermit crab, *Eupagurus longicarpus*, clots formed in at least two stages following injury—first a clumping and stickiness of hemocytes without change in shape or loss of granulation, then retraction of the clot and the development of a network of fibrous cell projections containing microtubules.

The abundant and elaborate literature on hemolymph coagulation in Crustacea was admirably summarized and evaluated by Florkin (1960). As he pointed out, coagulation has been considered by some authors to occur in two distinct phases—cellular coagulation and then plasma gelation—while other workers view coagulation as a continuous process in which plasma gelation begins around hemocytes. It was Florkin's

conclusion that plasmatic coagulation was a one-step process in which fibrinogen of the plasma was acted upon by a coagulin released by the hemocytes. It seems equally possible, however, that more than one type of coagulable protein exists and that the categories of clots may be complex rather than simple. Florkin also reviewed the role of cellular clots in wound repair, emphasizing the importance of secretion of a chitin film over the wound area by underlying coagulated phagocytes.

Encapsulation is also a common form of cellular internal protection in invertebrates. Invading organisms, often relatively large, are surrounded by phagocytes and fibrocytes. The onset of encapsulation may be rapid, and the cellular aggregates may be resolved only very slowly.

In summary, the hemocytes function in a number of ways beyond phagocytosis, although the latter must be considered the dominant cellular defense mechanism:

1. Hemocytes are important in cellular infiltration of injured or diseased tissue.
2. They are important in clotting—either as participants in cellular clots, or by release of secretions or injury products which combine with plasma components to form extracellular clots.
3. The hemocytes are of primary importance to encapsulation.

A great variety of crustacean hemocytes have been described during the past several decades. Animals studied included crayfishes (George and Nichols, 1948; Toney, 1958; Wood and Visentin, 1967), blue crabs (George and Nichols, 1948; Toney, 1958), lobsters (Toney, 1958; Hearing and Vernick, 1967), and brine shrimp (Lochhead and Lochhead, 1941). Except for size differences, the principal distinction seemed to be presence or absence of granules in the cytoplasm. The hyaline hemocytes are usually smaller than the granular, and some of the hyaline cells probably develop into the granular types, since the intergrades have been noted (Cuénot, 1895). As was aptly pointed out by Rabin (personal communication), "The developmental relationships of one form of hemocyte to another which have been made amount to little more than edu-

cated guesses, since they have been based largely on static images which may not even represent the true cell pictures as they occur *in vivo*." Both types of cells (hyaline and granular) probably have physiological subtypes, as suggested by the work of Fisher-Piette (1931) in which explants of lobster hemopoetic tissues resulted in multiplication of two types of hyaline cells—adhesive ameboid and non-adhesive non-ameboid. Inclusions of granular cells, in addition to their defensive function mentioned earlier, may also provide nutrient material—as suggested by release of this material into the hemolymph during ovarian development (Lochhead and Lochhead, 1941). Hyaline hemocytes may also be transformed into connective tissue or endothelial cells of blood vessels (Danini, 1925, 1927; Debaisieux, 1952a, 1952b; Demal, 1953). Hemocyte physiology and biochemistry are areas where additional studies are needed, but the extreme fragility of certain cells once they are removed from the normal animal has undoubtedly been a major deterrent.

Phagocytic activity has been ascribed in varying degrees to most recognized categories of hemocytes (Haeckel, 1862; Hardy, 1892; Cuénot, 1895; Bruntz, 1905, 1907; Kollman, 1908; Tait and Gunn, 1918; George and Nichols, 1948; Toney, 1958; Rabin, 1970b).

In recapitulation, the phagocytes of vertebrate and invertebrate animals have been investigated widely since the late 19th century, and the blood cells of Crustacea have received at least proportionate study. Cellular defenses of Crustacea and other invertebrates are varied but center on the phagocyte and its activities. Important also are the humoral defenses, which will be considered in the following sections. Before proceeding to considerations of other than cellular defenses, however, it seems relevant to include an often overlooked perimeter of defense suggested by Miles (1962). Early suppression of microbial numbers may be due to microbicidal activity of the tissue cells themselves, either innate or induced, or to soluble antimicrobial substances in the intercellular fluid of the integument. As Miles pointed out, such pre-inflammatory cellular defenses in no way diminish the importance of phagocytes and humoral factors, but only pro-

vide an added perimeter of defense. Antimicrobial capacities of tissues as a whole, and of nonphagocytic cells in particular, may be a mainstay of nonspecific resistance—in both primary invasion and the determination of subsequent courses of infection. Tissue defenses of this nature in the invertebrates may be of great significance.

HUMORAL DEFENSE SYSTEMS

Early studies of humoral factors in Crustacea produced significant, but at times ambiguous, results. Noguchi (1903) found that sera of lobsters and horseshoe crabs possessed natural agglutinins against various vertebrate erythrocytes. After repeated injections, he was able to demonstrate an induced hemolysin in the horseshoe crab but not in the lobster. Fredericq (1910), using a variety of antigens, was unable to demonstrate precipitins in a number of decapods (*Homarus vulgaris*, *Palinurus vulgaris*, *Carcinus maenas*, *Portunus puber*, *Cancer pagurus*, and *Astacus fluviatilis*).

The early literature on humoral mechanisms of internal defense in invertebrates, and especially the crustaceans, was clearly dominated by Cantacuzène and his students. Cantacuzène, in a period covering almost 3 decades beginning in 1912, examined the broad picture of humoral defenses of a large number of marine invertebrates. His work with Crustacea will be summarized in the next few pages as background for a consideration of subsequent studies.

Cantacuzène (1912a) reported (in a paper consisting essentially of a series of statements but little supporting data) the presence of natural agglutinins, lysins, and precipitins in serum of the hermit crab, *Eupagurus prideauxii*. Hemolysins for sheep and rabbit erythrocytes occurred in crab serum to a maximum titer of 250 and were destroyed by heat at 55° C. Agglutinins for rabbit erythrocytes persisted in dilutions beyond those at which hemolysis disappeared. Agglutinins for bacteria (*Escherichia coli* and *Vibrio cholerae*) were also present, as were weak precipitins against horse and rabbit sera. Cantacuzène also stated that comparable agglutinins, lysins, and precipitins were not present in *Pagu-*

rus striatus, which is closely related to *Eupagurus prideauxii*.

Cantacuzène (1923b), summarizing a decade of study of humoral defenses in invertebrates, found that the serum of the spider crab, *Maia squinado*, possessed natural agglutinins for mammalian red blood cells, with great individual variation from crab to crab. He observed, rather significantly, that agglutinins weakened or disappeared completely in crabs held in captivity for long periods. He noted, on injection of erythrocytes, that agglutinins disappeared completely during the first days after inoculation and did not return to their original titer for several weeks after the last dose. Cantacuzène also reported that rare individuals of *Maia*—invariably moulting females—possessed a lysin for mammalian red blood cells. *Maia* serum also possessed a strong lytic factor, but no agglutinins, against cholera vibrios.

Cantacuzène inoculated *Maia* with coelomic fluid of *Sipunculus nudus* (4 to 5 injections at intervals of 3 to 5 days) and found that the crabs produced first agglutinins and then lysins against the various injected sipunculid coelomic cells, including ova. The nature, intensity, and duration of response varied greatly among individuals. The lytic ability seemed more pronounced in females than in males, and more so in females approaching sexual maturity. Hemolysins against mammalian erythrocytes were also produced.

When he compared the lytic ability of *Maia* serum after injection with sipunculid fluid and mammalian erythrocytes, Cantacuzène found that response was more rapid and stronger with the former, and he concluded—on the basis of these and other studies—that mammalian red cells were only mediocre antigens for marine invertebrates. He attributed this weak antigenicity to coating of the injected mammalian erythrocytes in *Maia* and other invertebrates with a serum factor that interfered with subsequent reactions. Cantacuzène aptly referred to this as “mummification” of the red cells by invertebrate body fluids.

Concerning acquired immunity to bacterial infections in Crustacea, Cantacuzène (1923b) found that the crab *Maia squinado*, when inoc-

ulated with killed *Vibrio cholerae* or small doses of live vibrio, was able—12 days after the fifth injection—to survive the challenge with 20 times the dose of vibrio which had proved fatal to uninoculated control crabs.

Cantacuzène also reported on studies of the responses of *Maia squinado* to injections of gram-positive bacteria isolated from the crab's digestive tract. Inoculation was followed by reduction in numbers of amoebocytes and, within 24 hr, by disappearance of most of the bacteria from the hemolymph. The bacteria were immobilized in the various phagocytic tissues, particularly in the branchial lacunae—at first they adhered to the cell surfaces, then amassed into small granules, and were finally engulfed by fixed and mobile phagocytes. The process of digestion of bacteria was slow, and still incomplete after 7 weeks. Clotting ability of the hemolymph decreased immediately after inoculation but returned to normal in 8 days. No agglutinins for the bacteria could be demonstrated in vitro, but the natural agglutinins for mammalian red blood cells (discussed earlier) disappeared. In vitro studies disclosed that the hemagglutinin coated the bacteria but did not cause their agglutination. The adherence in vivo of the bacteria to fixed phagocytic cells undoubtedly was enhanced by the sensitization. Some evidence for this was gained by adding macerated hypodermal or pericardial cells to a mixture of bacteria and crab serum in vitro. The cell fragments acted as centers for bacterial agglutination and immobilization, but the agglutinating ability was not conferred on the serum by the addition of cell fragments.

A different sequence of events was described for those crabs (*Maia*) in which the experimental infections progressed to death. Early immobilization of bacteria in lacunar cells was followed, in 8 to 15 days, by the appearance of encapsulated forms, invulnerable to destruction by phagocytes. The encapsulated bacteria multiplied, phagocyte numbers were reduced, and the clotting ability of the hemolymph diminished. By 10 to 20 days after inoculation, the hemolymph became incoagulable, the natural agglutinin for red cells disappeared, the connective tissue became gelatinous, and the animal died.

Cantacuzène (1923b) also examined the internal defenses of the hermit crab, *Eupagurus prideauxii*, which he had reported earlier to possess strong hemolysins and strong antibacterial agglutinins. Injected gram-negative bacilli were entrapped and immobilized on the cell surfaces of the branchial lacunae, and then phagocytized, as was the case with *Maia* described previously.

Cantacuzène (1923b) also reported that the sera of crabs, *Carcinus maenas*, infected by the rhizocephalan *Sacculina*, contained a factor absent from normal crabs. Using a standard complement fixation test, with extract of the rhizocephalan as antigen and with crab serum, Cantacuzène (1925b) was able to demonstrate an antibody-like response in parasitized crabs. Sheep cells were lysed in tubes with normal crab serum but not in those containing parasitized crab serum. Using a fine suspension of *Sacculina*, Cantacuzène found precipitating and agglutinating activity in the serum of parasitized crabs. The activity was not consistent, however, in that some sacculinized crabs lacked it. In a concurrent study, Lévy (1923) found that macerated sacculinids were toxic when injected into crabs, but that no antitoxic activity could be demonstrated in parasitized crabs. Both groups, normal and parasitized, died at about the same rate.

In an earlier study Cantacuzène (1913) reported that inoculation of the sacculinid parasite with gram-negative bacteria resulted in septicemia and death of the parasite within 1 week, and infection of the crab host by 10 days after inoculation. At about 5 days after inoculation, the crab hemolymph became incoagulable, and agglutinins appeared against the bacteria inoculated into the parasite. The antibacterial agglutinins were not present in sacculinized uninoculated control crabs.

Appreciable evidence for some degree of specificity of the natural agglutinins of Crustacea was accumulated by Cantacuzène. An agglutinin in *Maia* against mammalian red cells could be absorbed from crab serum by certain gram-positive bacteria but did not agglutinate them, nor did it agglutinate cholera vibrios. It did, however, strongly agglutinate typhoid bacilli.

Agglutinins against vertebrate erythrocytes and certain bacteria were found in sera of *Homarus vulgaris*, *Eupagurus prideauxii*, and *E. bernhardus*, but the same antigens were not agglutinated by sera of *Cancer pagurus*, *Carcinus maenas*, *Portunus puber*, or *Galathea punctata*. The serum of *Eupagurus prideauxii* agglutinated mammalian red blood cells and amoebocytes of *Maia* and *Buccinum*, but did not agglutinate the coelomic cells of sipunculids or ascidians.

And so Cantacuzène set the scene, by the early 1930's, for the continuation of broad and elaborate studies of humoral internal defenses of marine invertebrates—particularly the Crustacea—but the stage, with only a few notable exceptions, remained curiously empty and dark until the mid-1950's. Although research during the last decade emphasized species other than those studied by Cantacuzène (except for the work of F. Bang), it reinforced many of Cantacuzène's findings: that natural agglutinins and lysins, with some specificity, occur in Crustacea and other invertebrates, and that responses to foreign antigens can be induced in selected invertebrates—responses which are only partially specific. The increased precision and quantitation of tests, and the careful attention to controls, have improved the quality of the newer data, but have neither provided new concepts nor modified the general conclusions of Cantacuzène. The more recent literature on humoral defenses of Crustacea will be summarized by general categories in the following sections.

Bactericidal Systems

Natural bactericidins have been reported from a number of marine invertebrates (Bang, 1967b). Recently, increase in titers of bactericidal activity after inoculation with Formalin-killed bacteria was noted in West Indian spiny lobsters, *Panulirus argus*, and American lobsters, *Homarus americanus* (Evans et al., 1968; Acton, Weinheimer, and Evans, 1969). A bactericidal assay system described by Schwab and Reeves (1966) was used to quantitate the degree of response. In experiments with American lobsters held in seawater at 5° C, the peak of

bactericidal response against gram-negative bacilli was reached within 48 hr after inoculation, and high titers persisted throughout the observation period (11 days). In spiny lobsters, presumably maintained at significantly higher environmental temperatures at Bimini, the Bahamas (reported as 26°-28° C in a later paper), the primary bactericidal response to intracardial injection of living or killed suspensions of the same gram-negative bacilli (originally isolated from the digestive tract of spiny lobsters) reached a peak at about 36 to 48 hr after injection, then declined slowly for the following 2 weeks. Partial lack of specificity of the bactericidin was indicated by its appearance following injection of gram-positive bacilli and by its activity against *Salmonella typhosa* and *Escherichia coli*, as well as against the unidentified gram-negative bacillus used as the homologous test organism. The bactericidin was not active, however, against *Pseudomonas aeruginosa* or against three species of gram-positive bacteria.

A subsequent study (Weinheimer, Acton, Sawyer, and Evans, 1969) of the specificity of the spiny lobster bactericidin further indicated that the response was partially nonspecific. Low titers of the bactericidin against gram-negative bacilli could be demonstrated after injections of Formalin-killed type 2 pneumococci and bovine serum albumin. Formalin produced a pronounced adjuvant effect.

Secondary responses—those following reinjection of the same antigen after a lapse of time—of spiny lobsters to killed suspensions of gram-negative bacilli were also examined by Evans, Cushing, Sawyer, Weinheimer, Acton, and McNeely (1969). Titers of bactericidin were slightly but significantly higher after reinoculation than after primary inoculation, and the rate of secondary response (the number of hours to reach peak titer) seemed somewhat accelerated when compared with the primary response. Unfortunately, the number of animals tested was small. It seems important that similar observations be greatly extended—since, as the authors pointed out, the results were reminiscent of the specific anamnensis or immunological memory demonstrable in the immunoglobulin responses of vertebrates.

The spiny lobster bactericidin was apparently a large molecule, as suggested by resistance to dialysis and by Sephadex separations. Inactivation occurred at 65° C and activity was not restored by addition of unheated normal hemolymph. Activity was not reduced by treatment with EDTA or carageenin. These results indicate dissimilarity with vertebrate complement-based bactericidal systems, but the authors suggested that the bactericidin may represent a primordial immunoglobulin.

Noteworthy is that all the American lobsters and a number of the West Indian spiny lobsters used in the studies by Evans, Weinheimer, Painter, Acton, and Evans (1969) had demonstrable pre-existing titers of bactericidins against the gram-negative bacillus used in the experiments. Possibly the remainder of the spiny lobsters used in the studies could have had titers of bactericidins lower than were demonstrable by the methods used. Thus inoculation may not have "induced" the bactericidin but instead may have merely enhanced or increased the titers. A number of explanations were offered for the pre-existing titers of bactericidal activity, including trauma because of handling, and response to previous bacterial infection. Two relevant observations are that in many American lobsters, a rapid increase in bactericidal titer preceded death, and in studies of spiny lobsters, the bactericidal activity was found to be partially nonspecific, in that activity against other gram-negative bacteria was enhanced.

The conclusions reached by the research group that examined the spiny lobster bactericidin (Weinheimer, Acton, Sawyer, and Evans, 1969) are in accord with findings for other phyla: "These data suggest that, although invertebrates appear capable of antigenic recognition, the molecules synthesized may have broad specificity covering a wide range of antigenic determinants. Further studies will be necessary to ascertain whether these inducible substances represent primitive immunoglobulins. In any event, it would be surprising if they did not have a major role in defense of the animal against pathogenic microbes."

An important qualification was pointed out by Aarum (1967) regarding results obtained by ex-

perimental inoculation of large numbers of bacteria. Infectious agents are found in large numbers in the circulatory system only in septicemia, which occurs only after a growth phase of a virulent pathogen within the host, often within fixed or immobilized phagocytic cells. Rapid increase in pathogen numbers in the circulating body fluid is usually a precursor to death and indicates a failure of host defenses. Thus the artificial introduction of large numbers of microorganisms should not be expected to elicit normal responses in an animal. It should also be noted that insusceptibility barriers to infection, operative in the normal animal, may be bypassed by experimental inoculation, resulting in massive infection and death from microorganisms not otherwise known as pathogens. A further qualification mentioned by Aarum is that even the simplest experimental manipulation may change phagocytic abilities, so that the normal phagocytic response of an animal to pathogens may be far different from responses elicited experimentally.

Lytic Systems

A natural hemolysin for sheep erythrocytes in the hemolymph of West Indian spiny lobsters was reported by Weinheimer, Evans, Stroud, Acton, and Painter (1969). Low temperatures (0° and 4° C) inhibited lysis, which was best demonstrated at 25° and 37° C. Heating the lobster hemolymph to 52° C destroyed lytic activity. The hemolysin could be adsorbed on red cells or cell stroma at low temperatures. Red cells with the adsorbed lysin were lysed when the temperature was raised to 37° C, which indicated a possible enzymatic type of activity of this hemolytic system. The authors suggested a multiple step system, analogous to mammalian hemolytic systems, consisting of a single protein species which is first adsorbed on the surface of the sheep erythrocyte, and then—in one or more steps—lyses the cell.

A detailed study of the presence or absence of natural immunity to invasion of the hemolymph of crabs by a parasitic ciliate, *Anophrys sarcophaga*, was reported by Poisson (1930). The ciliate, which in shore crabs, *Carcinus mae-*

nas, multiplies and causes death, was immobilized, agglutinated, and lysed in vitro by serum of several other crabs—particularly *Maia squinado*, *Eupagurus prideauxii*, and *Portunus puber*. Experimental inoculations of ciliates were cleared usually within several hours. It is interesting that the parasite, when experimentally introduced in another member of the Portunidae, *Portunus depurator*, multiplied and killed the experimental hosts just as it did in *Carcinus maenas*. Poisson attributed the reactions of crabs to the ciliate to expressions of a natural immunity, effected by agglutinating and lytic activity of the hemolymph.

Other lytic systems of Crustacea have received passing attention. Cantacuzène (1913) found that hemolymph of the hermit crab, *Eupagurus prideauxii*, possessed a heat-labile hemolysin, as well as precipitating and agglutinating activities. Cantacuzène (1921, 1923b) stated that injection of the spider crab, *Maia squinado*, with sheep erythrocytes produced hemolysins as well as agglutinins.

Agglutinating Systems

Natural hemagglutinins of the Australian freshwater crayfish, *Parachanna bicarinatus*, were examined by McKay, Jenkins, and Rowley (1969). Absorptions of hemolymph by erythrocytes of four mammalian and one avian species disclosed specificity, in that absorption by cells of one species still left agglutinins for cells of other species. The crayfish hemagglutinins were nondialysable and were inactivated at 57° C. In vitro studies with crayfish phagocytes and mouse erythrocytes disclosed that the crayfish hemagglutinins greatly enhanced the adhesion of the erythrocytes to the phagocytes—a specific opsonic effect. A similar effect was observed in vivo, apparently as a prelude to phagocytosis.

The recent studies support earlier reports of specific hemagglutinins in a number of invertebrate phyla (Tyler and Metz, 1945; Tyler, 1946; Sindermann and Mairs, 1959; Cushing, Calaprice, and Trump, 1963; Tripp, 1966) with specificities somewhat comparable to those of vertebrate natural isohemagglutinins and with some biological properties (such as enhancement of

adhesion and phagocytosis of erythrocytes by phagocytes) similar to those of vertebrate antibodies.

A study of natural agglutinins in the serum of California spiny lobsters, *Panulirus interruptus*, for blood and sperm cells of 54 species (representing 7 phyla) was published in 1945 by Tyler and Metz, and is still cited as one of the most comprehensive examinations of its kind for a marine animal. Agglutination was not found in 37 other species tested. Titers for positive reactions ranged from 8 to 256. The most interesting phase of the study was an extensive series of absorptions of spiny lobster serum by cells of many of the species tested. In each case, absorption of serum with cells of any single species removed agglutinins for all the species tested that belonged to the same group (Class) but left agglutinins for the cells of all other groups tested. Tyler and Metz concluded, on the basis of absorptions, that at least 10 class-specific agglutinins were present in the serum of the spiny lobster. A few cross-reactions occurred, and some reduction in titers resulted from absorptions with cells of other species, which suggested the presence of a number of reacting sites on the cells.

Bang (1967b) examined the responses of the spider crab, *Maia squinado*, to injections of *Anophrys*, a large ciliate pathogenic for shore crabs, *Carcinus maenas*, as part of a laudable attempt to repeat with modern methods some of the early French studies of invertebrate defenses. Sera of some spider crabs strongly agglutinated the ciliates, but that of others did not. Agglutination resulted from formation of a mucoid substance around the tail cilia. Crabs that lacked the agglutinin died from overwhelming infections of the hemolymph, whereas those with the agglutinin survived (Bang, 1962). When present, the agglutinin was apparently fairly constant, except that in some crabs it was lost spontaneously but temporarily at time of molting. Poisson (1930) had noted earlier that the hemolymph of *Maia* lysed the ciliates and that the hemolymph of the hermit crab, *Eupagurus prideauxii*, agglutinated them.

An agglutinin in the hemolymph of the hermit crab, *Paguristes ulreyi*, for human type O cells,

and to a lesser extent for types A and B, was reported by Cushing (1967). A number of individuals lacked the hemagglutinin, and some of these possessed a serum factor which inhibited the action in vitro of the positive sera. The analogy to specific soluble substances found in sera of certain vertebrates was pointed out by Cushing, with the suggestion that further studies of this kind with other invertebrates might prove instructive. Cohen (1968) found agglutinins for human and other vertebrate erythrocytes in sera from coconut crabs, *Birgus latro*. Young crabs lacked the agglutinins.

The serum of the American lobster contains strong and specific natural agglutinins for an antigen present on the red blood cells of sea herring, *Clupea harengus* (Sindermann and Mairs, 1959). Detection of blood groups in this fish was aided by the use of the hemagglutinin—individual herring either had the antigen or lacked it. Titers reached as high as 256, and reactions paralleled those obtained with absorbed rabbit antisera and plant lectins (Sindermann, 1963). Erythrocytes of other clupeoid fishes tested were also strongly agglutinated by lobster sera (Sindermann, 1962).

Precipitating Systems

Production of precipitins by invertebrates has been reported only rarely. Osawa and Yabuuchi (1963), in a very brief paper, found that the "Homard americain, *Cambarus clarkii*" [probably *Homarus americanus*] did not produce agglutinins or lysins when injected with red blood cells but did produce weak precipitins (detectable by immunoelectrophoresis) after injection with serum from rabbits and goats. No information was given about dosage, injection schedules, time for response, or titers.

Stewart and Foley (1969) suggested that a "precipitin-like principle already present in the hemolymph" of the American lobster might be important in removal of foreign protein. Fluorescein-labelled bovine serum albumin (BSA) and lobster serum proteins were injected into lobsters held at 5° C, and the fluorescence level checked periodically for 6 days. With lobster serum proteins, an initial decline in fluorescence

within 2 hr after injection was followed by a plateau that remained constant for the duration of the experiment; with labelled BSA, however, the rate of clearance was concentration dependent, but the foreign protein declined to very low levels by 3 days after injection. Fluorescent material was apparently excreted in proportion to its disappearance from lobster hemolymph. Pinocytosis by phagocytes was not demonstrated, but tiny granular fluorescent accretions were observed in the hemolymph of lobsters injected with labelled BSA, beginning about 8 hr after injection. In vitro studies, in which a standard ring test with labelled or unlabelled BSA and lobster serum was used, disclosed a clearly discernible ring after 16 hr at room temperature, and a precipitate at the bottom of the tube after 36 hr. Precipitin titers of individual lobster sera ranged from 2 to 16 and were independent of the total protein concentration of the lobster serum. Dialysis of lobster serum did not change the titers, but precipitin activity was destroyed by heating above 50° C.

A number of interesting implications in the study were pointed out by Stewart and Foley. The clearance mechanism seemed able to distinguish between foreign and native proteins, and the capacity for clearance seemed high, as indicated by accelerated clearance of larger doses of BSA. Attempts to increase the levels of precipitin by previous injection of lobsters with BSA did not succeed, and in fact resulted in decreased precipitin levels in some individuals. The authors suggested that the hemolymph factor responsible for clearance of foreign protein may be maintained normally at low levels and may be supplemented by further secretions when required. They also suggested that the precipitin principle in the hemolymph may be the first of several steps or possibly the primary removal factor and that digestion and excretion may take place elsewhere—probably in the hepatopancreas.

The results of the studies of Stewart and Foley are in agreement with those of Teague and Friou (1964), who observed that injected foreign protein was rapidly removed from the hemolymph of the crayfish *Cambarus virilis*. Previous injection of the protein did not increase the clear-

ance rate. Teague and Friou did not observe precipitin activity against injected bovine and human serum albumins but concluded that clearance resulted from nonspecific degradation of the foreign protein.

Other evidence of clearance of injected proteins but failure to induce heightened responsiveness in Crustacea was reported by Campbell and Garvey (1961). They mentioned that "It is also of interest that we have made many attempts to induce antibody formation in invertebrates, e.g., lobsters. We have been unsuccessful so far, but in every instance the antigens remained undigested and unchanged in the circulation and tissues for many months." Although not mentioned specifically, the lobsters were probably California spiny lobsters and the test antigens probably included BSA, since this was the principal antigen used in other studies reported in the same paper.

Phage Clearance

Taylor, Taylor, and Collard (1964) and Nelstrup, Taylor, and Collard (1968) presented some evidence (from two crabs) of an increase in the rate of secondary clearance of injected T₁ bacteriophage in the shore crab, *Carcinus maenas*. Clearance was not complete until after 2 weeks at 16° to 18° C, and no neutralizing antibody to T₁ phage was detected in the hemolymph. Primary inoculation with T₆ phage did not increase clearance rates for T₁ secondary injections. The small number of animals used in these experiments makes the conclusions highly tentative. The authors suggested the existence of a "phylogenetically more primitive type of immune response than the production of humoral antibody," but did not state clearly what the response was—except possibly that it was "an apparently purely cellular secondary response."

Studies of phage clearance by Cushing and McNeely (reported in Cushing, 1967) led to negative conclusions. Phage T₄ persisted for up to 168 days in the California spiny lobster and disappeared at a steady rate, uninfluenced by the size of the original inoculum. Two species of crabs tested also failed to clear bacteriophage.

Other negative findings for increased rate of phage clearance following inoculation in crayfish were reported by Teague and Friou (1964).

Antitoxic Activity

Little definitive information is available about antitoxic activity in invertebrates. As Huff (1940) pointed out, "Experimental demonstration of antitoxic action in invertebrates has failed for the most part because of lack of susceptibility of invertebrate cells for known toxins." Probably the best example of antitoxic phenomena in Crustacea was described by Cantacuzène (1925a) and Cantacuzène and Damboviceanu (1934a, 1934b). The hermit crab, *Eupagurus prideauxii*, exhibited resistance to nematocyst toxin of *Adamsia palliata*, a commensal coelenterate commonly found on the shell of the crab. When injected, the toxin had no effect on *E. prideauxii*, but it was lethal to many other Crustacea and to a number of other invertebrates tested, including the closely related hermit crab, *E. bernhardus*. Cantacuzène also found that serum of *E. prideauxii* could neutralize the coelenterate toxin when the two—serum and toxin—were mixed and injected into crab species susceptible to the toxin. The development of this antitoxic principle can be seen as a logical and necessary concomitant of the very close relationship of crab and anemone, but the question of whether this is an example of innate or acquired resistance has not been resolved.

Another example of coelenterate toxin lethal to crabs was reported by Lane, Coursen, and Hines (1961). Biologically active peptides in *Physalia* nematocyst toxins were tested, using fiddler crabs, *Uca pugilator*, as assay animals.

Except for the work with coelenterate toxins, evidence of antitoxins in invertebrates is weak. Stauber (1961) reported almost immediate removal of diphtheria toxoid from oyster blood, but Metchnikoff (1905) and Bengston (1924) found that tetanus and botulinus toxins remained in insect body fluids for several weeks without loss of toxicity. These studies must, of course, be viewed as most indecisive, since substances toxic to humans are not necessarily so to inverte-

brates. Reaction on the part of invertebrates could be identical to reaction against any other introduced foreign material.

Invertebrate responses to gram-negative bacterial endotoxins were the subject of a review by Levin (1967). The most striking activity of such endotoxins is the production, after experimental inoculation, of intravascular clots and the ensuing death of various crustaceans and other invertebrates. Antitoxic immunity has not been demonstrated, but, as Levin stated: "Endotoxin appears capable of activating complementary defense mechanisms in invertebrates, including aggregation of amoebocytes, coagulation, bacterial immobilization, and phagocytosis. All these may be operative through one type of cell—the amoebocyte."

Other Protective Systems

McKay and Jenkin (1969) examined resistance of the Australian freshwater crayfish, *Parachanna bicarinatus*, to a pathogenic *Pseudomonas* sp. and concluded that the animal was capable of an adaptive immune response. Their findings indicated lower mortality rates (after bacterial challenge) in animals inoculated with heat- and alcohol-killed vaccines as well as with endotoxin. Inoculation with vaccines prepared from other gram-negative bacteria also increased the level of resistance to the *Pseudomonas* infection, but vaccines from gram-positive bacteria did not—indicating some degree of specificity. A positive correlation was found between survival of challenged animals and the number of exposures to bacterial antigen; after four inoculations, the LD₅₀ of immunized animals was nearly 100 times that of controls. Temperature also played a significant role in the onset, degree, and duration of protection induced by inoculation of animals with killed bacteria. At 26° C, onset of protection was rapid (1 day), reached a peak at 3 days, and almost disappeared by 12 days; at 19° C, onset was slower (2 days), reached a maximum at 4 days, and persisted for 12 days (the duration of the experiment); at 14° C, no protection was afforded. Inoculation of gram-negative endotoxin resulted in protection similar in appearance and duration to that

produced by killed vaccines. Although the terms "immunity" and "resistance" were used, the precise nature of the protection afforded by inoculation of vaccines and endotoxin was not described by the authors. In vitro experiments with hemolymphs of control and resistant crayfish disclosed no bactericidal or bacteriostatic effects, and McKay and Jenkin suggested that the most important effect of immunization may have been to increase the metabolic rate of the phagocytes [thereby stimulating phagocytosis].

Barker and Bang (1966), extending the earlier studies of Cantacuzène (1925b) with the shore crab, *Carcinus maenas*, and its rhizocephalan parasite *Sacculina carcini*, reported that inoculations of *Vibrio* sp. caused the hemolymph of the parasite to become incoagulable within 24 hr. Masses of gelled material containing bacteria were seen within body spaces. Septicemia and death, first of the parasite, and then often of the crab host, followed soon after.

Insusceptibility factors seem operative when certain parasites of invertebrates fail to develop. Michajlow (1938) and Baer (1944), for instance, found that larval cestodes, *Triaenophorus* and *Ligula*, penetrated the intestinal wall of a number of copepods, but developed only in certain species. In others, the larvae died and were phagocytized. Hedrick (1935) observed similar differences in survival of larval nematodes. Léger and Duboscq (1908) reported earlier that sporogony of the sporozoan *Agregata eberthi* (which occurs in the intestinal wall of crabs of the genus *Portunus*) took place readily in all species except *P. puber*, in which the parasite was quickly phagocytized after invading the intestinal wall.

INTERNAL DEFENSE MECHANISMS INVOLVED IN GAFFKAEMIA OF LOBSTERS

The American lobster, *Homarus americanus*, has an effective internal defense system, consisting of active phagocytosis as well as agglutinating and bactericidal (or bacteriostatic) activity, against a number of injected bacteria. The protective system seems to fail completely only

when challenged by *Gaffkya homari*—which is thus far the only bacterial pathogen known to develop systemic infections in lobsters and to kill them. Probably the most extensive series of reports concerned with responses of invertebrates to a particular pathogen is that dealing with the lobster (and other decapods) and the highly pathogenic gram-positive micrococcus *G. homari*. "Gaffkaemia"—the disease caused by *G. homari*—is enzootic in both the American lobster, *Homarus americanus*, and the European lobster, *H. vulgaris*, and has been reported to cause epizootics in captive populations of both species (Roskam, 1957; Goggins and Hurst, 1960; Gibson, 1961; Stewart and Rabin, 1970). Microorganisms with characteristics of *G. homari* have been isolated from shrimp (*Penaeus aztecus* from the Gulf of Mexico) and from crabs (*Carcinus maenas* and *Libinia emarginata* from New England and *Cancer irroratus* from eastern Canada), but the disease "gaffkaemia" is known only in lobsters. Early descriptions of the disease and its etiological agent (Hitchner and Snieszko, 1947; Snieszko and Taylor, 1947) have been followed during the past decade by studies in several laboratories, which used the lobster and the pathogen as a test system to elucidate responses to infection and other aspects of the host-parasite relationship. The possible course of infection in lobsters is summarized in Table 1. Snieszko and Taylor (1947) first satisfied Koch's postulates for the pathogen and demonstrated high mortality following inoculation of cultured *G. homari*. Stewart and MacDonald (1962) and Stewart et al. (1966) found that 40 to 60% of lobsters they examined from certain locations on the Canadian east coast were infected.

Studies by Harvey Rabin at Woods Hole and The Johns Hopkins University (Rabin, 1965; Rabin and Hughes, 1968) confirmed that lobsters inoculated with *Gaffkya* became septicemic within 2 days and died a few days later. Inoculation of gram-negative endotoxin 10 hr before exposure to the pathogen did not alter the course of infection. Prior inoculation of heat-killed *Gaffkya* cultures (24 hr before challenge) produced no protection.

In vitro studies with lobster serum as a culture medium disclosed that *Gaffkya* growth was

TABLE 1.—A proposed hypothesis to explain the course of *Gaffkya homari* infections in lobsters at 15° C.

Day	Development of gaffkaemia in lobsters
0	Bacteria gain access to tissues of lobsters as a result of injury which destroys the integrity of exoskeleton (or possibly the gut epithelium). Lobster hemocytes phagocytize <i>G. homari</i> .
2	Phagocytized bacteria may multiply within hemocytes. Hemocytes containing engulfed bacteria lodge in capillary and lacunar areas (heart, hepatopancreas, gills) of the lobster.
4	Hemocytes may be disrupted, releasing <i>G. homari</i> in hemolymph. This may result in rapid decrease in hemocyte numbers and logarithmic increase in bacterial numbers.
6	Hemolymph stimulates multiplication of released bacteria. Hemocyte numbers seem to be gradually reduced by continued phagocytosis and disruption of phagocytes.
8-10	Clotting mechanism (release of coagulin from hemocytes) is affected—possibly by reduction of hemocyte numbers—and clotting time is greatly prolonged.
12-14	Lobsters die from depletion of nutrient stores and utilization of this material by <i>Gaffkya</i> . Injured gaffkaemic lobsters may bleed to death. (The possibility of exotoxin has not been entirely eliminated, but there is no present evidence to suggest its existence.)

stimulated, while growth of a *Vibrio* (nonpathogenic to lobsters) was usually inhibited. Serum from lobsters which had been inoculated 24 hr earlier with killed *Gaffkya* still stimulated growth of the pathogen in vitro.

Rabin and Hughes (1968) tested resistance to *Gaffkya* in a variety of studies with lobsters and other marine arthropods. Findings with spider crabs (*Libinia emarginata*), rock crabs (*Cancer borealis*), and horseshoe crabs (*Limulus polyphemus*) were that most of the test animals cleared inoculated *G. homari*. In vitro studies with hemolymph disclosed either no apparent effect or only slight inhibition of growth of the pathogen by sera of spider and horseshoe crabs, and a slight stimulation of growth by sera of rock crabs.

The possible role of exotoxin was tested in lobsters by Rabin and Hughes with inoculation of filtrates of *G. homari* cultures. The filtrate had no effect when it was injected into the abdomen, but injection into the major joint of the chela induced autotomy or abnormal movements in over 50% of the lobsters treated.

Evidence of resistance to gaffkaemia was noted by Rabin and Hughes in a single lobster, which had been infected naturally before it was brought to the laboratory. Twelve days after

capture the lobster was free of the pathogen. The animal was inoculated twice with increasingly larger dosages of *G. homari* and cleared the bacteria within 6 days—but died on the 11th day following the second challenge. The reactions indicated a partial resistance and an ability in some individuals to recover from gaffkaemia. It is interesting that serum from this presumably resistant lobster was similar to that of other lobsters tested in that it did not inhibit growth of *G. homari* in vitro.

Rabin and Hughes stated that the presence of *Gaffkya* infections did not damage the clotting mechanism—an observation quite different from that of Goggins and Hurst (1960), who found that reduction in amebocytes and a much prolonged clotting time were distinctive features of the disease. Stewart et al (1969) and Stewart and Rabin (1970) clarified these seemingly disparate observations by reporting that “coagulin” is released to initiate clotting by rupture of hemocytes and that the “concentration of plasma proteins, including fibrinogen, does not appear to decline significantly in gaffkaemic lobsters.” An earlier report by Rabin and Hughes (1968) stated that when extract of lobster muscle was used as a coagulin source, recalcified clotting times were the same in diseased and normal animals. When these facts are combined, it can be concluded that the abnormally and persistently low hemocyte content of the hemolymph results in prolonged clotting time and does not indicate any deficiency in plasma constituents other than coagulin (Figure 3).

Studies carried on by James Stewart and his associates at the Halifax (Nova Scotia) Laboratory of the Fisheries Research Board of Canada have extended the work of Rabin and have provided the greatest number of contributions to the literature about the effects of *Gaffkya* disease on lobsters. In accord with earlier studies, infections usually were fatal, although rare individuals infected with *Gaffkya*-like organisms did survive (Stewart et al., 1966).

Cornick and Stewart (1968a) provided considerable relevant information about the host-parasite relationships of *Gaffkya* and lobsters. Experimental infections by inoculation, in which dosages as low as approximately 5 bacteria per

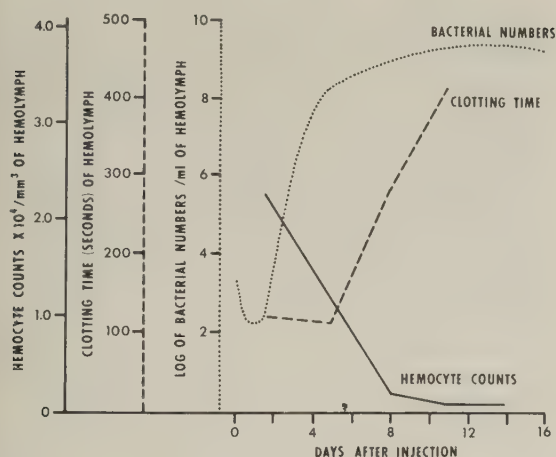


FIGURE 3.—Relations of hemocyte counts, clotting time, and bacterial numbers to time from experimental exposure of lobsters to *Gaffkya*. (Redrawn from figures in Stewart, Arie, Zwicker, and Dingle (1969) and Stewart and Rabin, 1970.)

lobster at 15° C were used, killed 90% of the test animals within 17 days. The absence of an effective host defense against *Gaffkya* was strongly indicated by the fact that the mean time to death was almost constant, regardless of dosage (Figure 4). Cornick and Stewart's studies disclosed additional facts that help to explain the pathogenicity of the bacterium to lobsters: *Gaffkya* resisted digestion in phagocytes and multiplied in the hemolymph; growth of *Gaffkya* was stimulated in vitro by serum of lobsters while growth of several other bacteria was inhibited; and *Gaffkya* was not agglutinated by lobster serum, though all other bacteria tested were agglutinated.

Presence in lobster hemolymph of effective defenses against bacteria other than *G. homari* was indicated by clearance within 30 days of inoculated suspensions of *Micrococcus conglomeratus*, *M. sedentarius*, *Achromobacter thalassius*, and *Gaffkya tetragena*. Since several of these bacteria are closely related to *G. homari* (which was not cleared), some specificity of the phagocytic or humoral protective mechanisms is strongly indicated.

Natural agglutinins in lobster serum were demonstrated against all bacteria tested (six

genera), except for all strains of *G. homari*. Such agglutinins were of low titer, nondialyzable, inactivated at 56° C, and seemed to be non-specific (as suggested by the limited observation that a single absorption of serum by *Flavobacterium marinum* removed agglutinins for all other bacteria tested except *Brevibacterium* sp.).

Cornick and Stewart's observations on phagocytosis of *G. homari* are interesting and warrant further investigation. They found no phagocytized bacteria in hemolymph preparations 15 min after inoculation, but they did find fluorescent-dye-labelled bacteria in hemocytes in heart, liver, and gill tissues of experimental lobsters soon after inoculation. Circulating hemocyte numbers were reduced significantly within 15 min after bacterial inoculation but returned to normal levels after 5 hr. These data are in agreement with the statements of Maynard (1960), that phagocytes which have engulfed foreign material lodge in capillary and lacunar areas of the crustacean body, resulting in reduction in numbers of circulating hemocytes. Bang (1956) observed in tissues of *Limulus* injected with gram-negative bacteria a similar reduction in circulating hemocytes. In Cornick and Stewart's study long-term infections of lobsters were characterized by the presence of black nodules containing *G. homari* in tissue cells in the gills, swimmerettes, and ventral abdominal

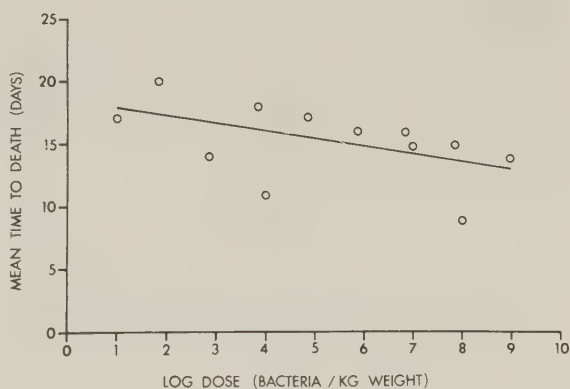


FIGURE 4.—Relation of dosage of *Gaffkya* to mean time to death (MTD) in lobsters (calculated line of best fit for mean time to death, using experimental groups of 10 lobsters each). (From Cornick and Stewart, 1968a.)

sinuses of the lobster. As Parry (1960) had pointed out earlier, this type of aggregation in gills is a common phenomenon in Crustacea. Poisson (1930) observed that, in the few crabs (*Carcinus maenas*) resistant to the parasitic ciliate *Anophrys sarcophaga*, masses of dead ciliates occurred in branchial lacunae, pericardial sinus, and hepatopancreatic sinuses. Degenerating ciliates eventually formed brownish cysts. Cantacuzène (1923b) observed a similar phenomenon in *Maia squinado* inoculated with bacteria. He pointed out that the lacunar tissue of the branchial lamellae of decapod crustaceans, with its many fixed phagocytes, acts as an extensive and effective bacterial filter.

Cornick and Stewart suggested that the development of polysaccharide capsules by *G. homari* in later stages of infection could be an important device that prevented destruction of the phagocytized bacteria and allowed multiplication of the pathogen. They pointed out, as evidence, that unencapsulated *G. homari* grown in culture were actively phagocytized. As Stewart and Rabin (1970) later reported, however, the unencapsulated cultured bacteria were also virulent. It may be that the capsule forms soon after the organisms are injected into the host. This observation of survival and growth of phagocytized encapsulated bacteria in lobsters is a direct counterpart of the inability of vertebrate phagocytes to destroy many encapsulated microorganisms, and parallels the earlier findings by Cantacuzène (1923b) of a fatal disease in crabs induced by encapsulated gram-positive bacteria.

Stewart, Dockrill, and Cornick (1969) examined certain insusceptibility factors affecting *Gaffkya* disease in lobsters. Destruction of the integrity of the integument seemed essential to transmission of the pathogen. Acidity of the gastric fluid was bactericidal and appeared to provide an effective barrier against oral infection. Previous attempts to infect lobsters by feeding infected material had been unsuccessful (Snieszko and Taylor, 1947; Wood, 1965a, 1965b; Rabin and Hughes, 1968).

Undoubtedly such insusceptibility factors are of definite importance to the epizootiology of gaffkaemia for several reasons: lobsters are cannibalistic; hemolymph of moribund gaffkae-

mic individuals contains about 10^9 organisms per ml (Stewart, Arie, Zwicker, and Dingle, 1969); and *Gaffkya* can be isolated consistently from lobster pounds, sea water, bottom mud, and slime of holding containers (Goggins and Hurst, 1960).

A thorough study of the effects of temperature on experimentally induced *Gaffkya* infections in American lobsters was reported by Stewart, Cornick, and Zwicker (1969). Mean time to death was inversely related to temperature (Figure 5). At 1°C no deaths attributable to experimental infections occurred; at 3°C mean time to death was 172 days; and at intermediate higher temperatures mean time to death decreased drastically to a minimum of 2 days at 20°C (which approaches the upper lethal tem-

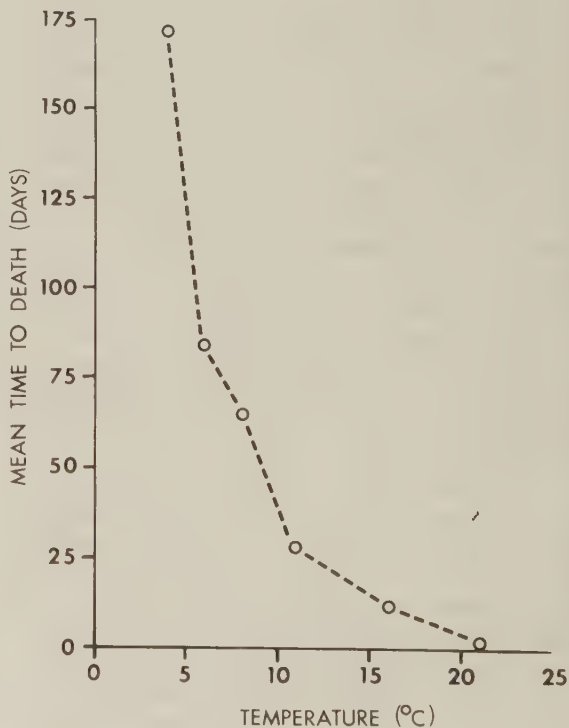


FIGURE 5.—Relation of temperature to mean time to death in lobsters experimentally exposed to *Gaffkya*. (From Stewart, Cornick, and Zwicker, 1969.)

perature for American lobsters). It is important to note (in view of the very low seasonal temperatures of waters in which lobsters live naturally) that at 1° C the pathogens persisted in the host in low numbers but with virulence unchanged, and they produced mortalities when the temperature increased. Experimentally infected lobsters were also sensitive to and died from rapid increases or decreases in environmental temperatures—although the temperature changes used in the experiments were probably greater than those that would normally be experienced in nature.

Findings in vivo were paralleled by in vitro results of growth of *Gaffkya* in lobster serum—with a more rapid increase to a peak of bacterial numbers with increasing temperature. The organism grew in culture at all the experimental temperatures (within a range of 1° to 20° C); at 1° C the bacterial growth curve was erratic—it decreased in numbers to the 30th day, then a log increase progressed to the 60th day, followed by a substantial decline. The important observation, of course, is that *G. homari* can survive within the range of environmental temperatures experienced by American lobsters and that the pathogen causes mortality more rapidly as temperature increases.

A concurrent physiological and biochemical study by Stewart, Arie, Zwicker, and Dingle (1969) and Stewart, Foley, and Ackman (1969), in which an attempt was made to define features of the infection that lead to death of lobsters, produced several interesting results. The pathogen lacked proteolytic, lipolytic, and fibrinolytic exoenzymes, suggesting that harmful effects are not caused by direct destruction of tissue. The authors observed that although in vitro growth of *G. homari* was limited by the carbohydrate level of the lobster serum medium used, presumably such a level would be maintained in vivo at the expense of other tissues. Drastic reductions in hepatopancreatic glycogen and hemolymph nonprotein nitrogen characterized later stages of the infection. No evidence of a toxin was detected, and the conclusion was that gaffkaemia is largely a wasting type of disease—that death from the disease was “a result of an unsuccessful competition on the part of the

lobster for its own readily available storage material.”

On the basis of experimental inoculations and subsequent mortalities, Bell and Hoskins (1966) suggested that *Gaffkya* might be pathogenic for the Dungeness crab, *Cancer magister*, and the shrimp *Pandalus platyceros* from the Pacific coast. That observation could be important in view of recent introductions of American lobsters (some possibly carrying *Gaffkya*) on the Canadian west coast. Other experimental studies (Cornick and Stewart, 1968b) indicated that the bacterium may also be pathogenic for east coast crabs (*Cancer irroratus*, *C. borealis*, and *Hyas coarctatus*). In vitro growth of the pathogen in crab sera was similar to that in lobster serum, suggesting susceptibility of the crabs. However, agglutinins for *G. homari*, which were demonstrated in the sera of one of the crab species (*C. irroratus*), might counteract the favorable bacterial growth in vivo and reduce the severity of infections in the crab. Cornick and Stewart extended their observations by inoculations of *C. irroratus* with suspensions of *G. homari*. After 49 days the surviving crabs (three) were found to be heavily infected (10⁹ organisms/ml hemolymph). Passage through the crabs did not alter pathogenicity of *Gaffkya* to lobsters. A repetition of the crab inoculations with larger numbers of crabs provided some evidence of greater mortalities in experimental groups than in controls. Pathogenicity for rock crabs was less than for lobsters, as indicated by a mean time to death of 42 days in crabs, against only 18 days in lobsters. The authors mentioned the possible role of rock crabs as reservoirs of infection for lobsters, in view of reduced pathogenicity and prolonged mean time to death in crabs.

From the foregoing, it is apparent that experimental studies with *G. homari* have been numerous and varied and have provided significant insights about the internal defenses of Crustacea. Important areas for future study include determination of whether strains of the pathogen with different virulences exist, and determination of whether virulence may be increased by rapid passage through impounded lobster populations.

DISCUSSION

In the development of information and principles of invertebrate internal defenses, consistent and entirely natural attempts have been made to translate findings into the concepts and compartments constructed for the immune responses of vertebrates. The effort has led to some confusion of terminology and even to lack of agreement about definitions of immunity.

The concept of immunity in vertebrates has been admirably stated by Good and Papermaster (1964), who define immunity precisely and narrowly as "a biologic phenomenon embodying primary and secondary responses, with antibody synthesis and release, reactions of immediate and delayed allergy, and homograft immunity." They state: "To the time of writing [1964], adaptive immunologic responsiveness has not been demonstrated in the invertebrates." They then define adaptive immune responsiveness as "the ability to respond to antigenic material by production of specific combining substances, and to show an anamnestic response to these same antigens on subsequent exposure." Their continued exposition of immunity from the vertebrate point of view includes the following significant points:

1. "Adaptive immunity . . . is primarily a function achieving full expression late in phylogeny and ontogeny."

2. "The lymphoid cell family is the primary cellular basis for adaptive immune response in vertebrates"

3. "The possibility that another cell system may mimic adaptive immune responses in an invertebrate species cannot be excluded at this time."

A broader, more inclusive, concept of immunity has been suggested recently. If the broader definitions of terms proposed by several authors are accepted, the words "immunity" and "immune response," rather than careful circumscriptions, can be used with invertebrates. As an example, McKay and Jenkin (1969) stated that the Australian freshwater crayfish was capable of an "adaptive immune response." Such a capability is not possible within the confines

of Good and Papermaster's definition of adaptive immunity as the production of specific immunoglobulins (a capacity which has been correlated with the occurrence of lymphoid tissue). Perhaps their definition is too restrictive and rigid, since a number of invertebrates do show responses that protect them from pathogens (hence they are adaptive).

Earlier definitions of antibodies and immunity allowed more latitude for inclusion of invertebrate responses. Cantacuzène (1923b), for example, considered as antibodies ". . . toute substance albuminoïde du plasma, douée ou non de spécificité, qui, se fixant sur l'antigène, modifie les relations de contact de ce dernier, soit avec les cellules, soit avec les autres constituants chimiques des humeurs."

McKay, Jenkins, and Rowley (1969) stated ". . . to allow comparisons to be made between invertebrate phyla and the vertebrates . . . the definitions of the immune response should be as broad as possible and emphasis placed on the functional aspects" These authors suggest that such a definition of the immune response might be "the ability of the animal to respond to a foreign particle (whether it be truly foreign or unwanted self) by the production of specific proteins capable of reacting with the inducer, and the resultant of this reaction leading to phagocytosis."

Cushing (1967), in an excellent summarization of invertebrate immune mechanisms, stated "There is a growing consensus of observations supporting the view that while vertebrates and invertebrates may share some basic immune competences such as 'innate immunities' and phagocytic cells, it is indeed only within the vertebrates that the full capacity of adaptive immunity exists." This statement seems reasonable, and fits the confines of Good and Papermaster's narrow definition of adaptive immunity, but is too negative if a broader perspective of immunity—such as that proposed by McKay, Jenkins, and Rowley—is adopted.

Probably greater emphasis should be placed on the *adaptive* aspects of invertebrate internal defense processes. Substantial numbers of studies have indicated the existence of adaptive responses to experimental inoculations of for-

eign protein. Whether the response is in the form of specific immunoglobulin seems less significant than the degree of protection afforded to the individual by the adaptive response. In the broadest sense, the replacement of a protective constituent of the hemolymph after its utilization in preventing infection could be considered adaptive. For example, the precipitating factor for foreign protein found by Stewart and Foley (1969) in lobster serum (which decreases following experimental inoculation of BSA and which is unreactive against injected lobster serum) would be adaptive.

Considering immune responses of vertebrates and invertebrates, Good and Papermaster stated that the presence in vertebrates of lymphoid tissue and cells constitutes a basic distinction. On this basis, as Chadwick (1967) has pointed out, "It is highly unlikely that insects [or Crustacea or other invertebrates] do produce mammalian type antibody, or that the mechanism of any acquired response to antigenic stimulus could be likened to responses in higher animals in terms of the production of specific antibody globulins." Analogous tissues and cells exist in a number of invertebrate groups, however, as do analogous humoral responses without the extreme specificities of vertebrate globulins. Chadwick (1967) also stated that the "... immune response in an insect is not the consequence of an antigen-antibody-globulin reaction but more likely the result of the production of some, as yet undefined, principle in insect hemolymph which may contribute to its resistance." The same statement might be made about other invertebrates in which an induced response has been demonstrated.

Although somewhat beyond the confines of the present consideration of internal defenses of Crustacea, it might be well to call attention to recent tissue transplantation work of Cooper (1968, 1969a, 1969b, 1969c, 1969d) with annelids, which indicates a high degree of specificity of response and which suggests some similarities to vertebrate tissue graft responses. Cooper's (1969d) concluding statement is significant: "Further clarification of anamnestic responses to tissue transplants would confirm our views that at least two of the parameters of

adaptive immunity [in the vertebrate sense], namely specificity and memory, did not evolve exclusively with the lower vertebrates."

It is obvious that modification, redefinition, or replacement of some conventional immunological terminology—particularly toward broader definitions—is needed if the invertebrates are included in comparative immunology. If we remove "antibodies" from invertebrate terminology we must also remove "antigen," since antibody response is part of the definition of antigen. An effective substitute for "antigen" (as suggested for insects by Hinton) (Chadwick, 1967) would be "immunogen." Chadwick also suggested replacement of "antibody" with such terms as "natural bactericidal substance," "specific inducible substance," and others. Furthermore, as stated earlier, it must be made clear that when "lysins," "precipitins," "agglutinins," and other humoral factors of invertebrates are discussed, identification with vertebrate factors is not intended—the terms are used merely to indicate the kind of activity produced (i.e., "lytic substance or activity," "precipitating substance or activity," etc.) regardless of the physiological-biochemical mechanism(s) involved.

When suitably qualified the "safe" general terms, therefore, include "resistance," "immunity," "immune response"; terms that can have general applicability and utility, if accepted in a general sense, include "agglutinin," "lysin," "precipitin"; specific vertebrate terminology, not applicable to invertebrates includes "antibody," "antigen," and "serological."

Beyond the establishment of working definitions of immunity in invertebrates, it seems appropriate to list a number of generalizations that seem warranted by the admittedly narrow base of evidence now available. Obviously, any generalization about a group as evolutionarily diverse as the invertebrates—or for that matter even of the Crustacea—must be in the form of a tenuous and easily retractable hypothesis (which may at times border on speculation), and the following statements are offered with these qualifications:

1. Resistance in the vertebrates seems primarily related to production of immunoglobulins

which combine with foreign protein to enhance the phagocytic process. Although specific immunoglobulins have not yet been demonstrated in invertebrates, an analogous protein system, of lower specificity but with functions similar to vertebrate immunoglobulins, is suggested. Natural (and in some cases, partially specific) agglutinins are common in invertebrate body fluids, and their titers in some species may be increased by exposure to specific antigens.

2. Resistance in the vertebrates, and in some invertebrates also, includes the production of bactericidins, lysins, and agglutinins. The appearance of, or the increase in, titers of such factors in certain invertebrates following exposure to foreign proteins may account in part for increased resistance to certain pathogens (McKay and Jenkin, 1969; Bang, 1967b).

3. Many of the bactericidal, bacteriostatic, lytic, and agglutinating properties seem to be conferred on the hemolymph of invertebrates by release of materials from hemocytes. The substances so released often seem not only less specific in their action than vertebrate antibodies, but also the stimulation of release may be much less specific. For example, the release of a lysin in sipunculids for the ciliate *Anophrys* can be stimulated by inoculation of certain bacteria (Bang, 1967a), and the release of a hemolysin in *Maia squinado* for sheep red blood cells can be induced by injection of sipunculid coelomic fluid (Cantacuzène, 1920a, 1923b).

4. Immune response in invertebrates, as best exemplified in insects and crustaceans, is often rapid in onset and disappearance—usually a matter of a few days.

5. As has been observed by a number of authors (Feng and Stauber, 1968; Stewart, Cornick, and Zwicker, 1969), environmental temperature is a critical factor in the host-parasite relationships of invertebrates. Temperature has been found experimentally to exert a significant effect on the appearance, degree, and duration of resistance to infection in certain invertebrates (McKay and Jenkin, 1969), just as it does in poikilothermic vertebrates (Bisset, 1946, 1947a, 1947b, 1948a, 1948b). Temperature affects the rate of growth and reproduction of microorganisms, the rate of production of

toxic metabolites, and the utilization of nutrient derived from the host. Temperature can also affect the rate of phagocytosis and the rate of production of humoral defenses against infection. Thus the progress of infection and the outcome of disease represents a composite of enhancement or inhibition partly mediated by temperature.

6. Endotoxin has been found (in the vertebrates) to increase the metabolic rate of phagocytes and stimulate phagocytosis (Jenkins and Palmer, 1960; Whitby et al., 1961). A similar effect may be produced by endotoxin in the invertebrates. Thus, exposure to gram-negative bacteria which are so abundant in the sea (or to their endotoxins) may increase the level of nonspecific resistance to other gram-negative organisms or their endotoxins. This "nonspecific immunity," which is also known in the vertebrates (Rowley, 1956; Landy and Pillemer, 1956), may be of great significance in the invertebrates—in fact, it may be the basic mechanism of internal resistance to bacterial pathogens in the invertebrates.

7. Handling and experimental procedures rapidly induce bacteremias in a number of invertebrates. Rabin (1965), for example, found that almost half of all American lobsters used in his studies had bacteremias upon arrival in the laboratory. Cornick and Stewart (1966) found that about 20% of a large sample of lobsters had bacteria in their hemolymph. Isolates were principally *Micrococcus*, *Pseudomonas*, *Brevibacterium*, and *Achromobacter*—bacteria commonly found in the marine environment, and apparently nonpathogenic for lobsters. The acts of capture, transport, and impoundment of these and other marine animals may produce stresses and physiological changes (or mechanical damage) that permit entry of microorganisms common in the surrounding sea water.

8. There is some limited evidence that the process of phagocytosis, fully elaborated in the Protozoa, is enhanced in the vertebrates by non-specific humoral factors which sensitize, agglutinate, immobilize, or otherwise increase the susceptibility of proteins to phagocytosis by fixed and mobile phagocytic cells. Although the opsonizing substances of invertebrates have not

been adequately characterized and the mechanisms involved have not been adequately elucidated, it may be speculated that in the vertebrates a greater degree of specificity of humoral factors has been added to the nonspecific mechanisms found in the invertebrates.

The proper role of specific vertebrate antibody as a possible augmentation of evolutionarily older nonspecific internal defenses was alluded to by Miles (1962). He stated "... it is fairly clear that antibody *per se* has little effect on the viability or metabolism of microbes with which it combines. It is effective in defense either because it neutralizes toxins, or because it makes the microbe susceptible to non-specific defense factors like complement or the phagocyte We may then properly consider antibody as accessory to the more fundamental nonspecific defense mechanisms" It should be emphasized, however, that much remains to be learned about the nonspecific humoral factors of vertebrates, as well as invertebrates.

9. Brown (possibly chitinous) bodies or cysts in gills characterize later stages of a number of crustacean diseases. The sequence of events, after invasion by microorganisms, may include action of toxic or inhibitory factors in hemolymph, accretion of moribund or dead invaders in gill lacunae, phagocytosis of dead organisms, and formation of nodules or cysts containing dead organisms, and gradual phagocytic destruction of necrotic material.

10. An important point, as Bang (1967b) mentioned, is that the probability of discovering internal defense mechanisms is greater when disease phenomena are studied under natural conditions. In experimental work, microorganisms pathogenic to marine invertebrates should constitute test organisms of choice; microorganisms found in the environment (and which may be facultatively pathogenic) should be next in order of preference; and microorganisms or proteins which the marine animal is unlikely to encounter seem least instructive. There are valid experimental reasons, of course—such as the ease of recognition of bacteriophages—that often lead to selection of test microorganisms other than pathogens or potential pathogens. Whether

these unusual choices are effective antigens is obviously a most important consideration.

Another extremely pertinent observation made by Bang (1967b) was: "The limited amount of information [concerning immunological responses of invertebrates] is, I believe, due mainly to the limited number of studies, and not to any lack of imagination on the part of evolutionary forces in developing protective mechanisms."

11. One final and very significant thought was proposed by Stauber (1961): "That so few examples of acquired resistance are known among invertebrates may even be quite logical. Because of their relatively short generation times, their usual small size and often enormous reproductive capacities, subsequent epizootics would be much more likely to be circumvented by the appearance of resistant stocks through natural selection Even with very high mortality rates a residual stock of animals under favorable conditions later might repopulate an area If this reasoning is adequate to explain the lack of evidence for the occurrence of acquired resistance in most of the invertebrates, perhaps those invertebrates with a long life span, like *Limulus* should be investigated more fully, as likely hosts capable of demonstrating acquired resistance."

It is interesting to note that it is precisely those invertebrates with a long life span which have received increased attention during the past several years, and that a few indications of acquired resistance have been reported.

Information about the internal defenses of crustaceans and other invertebrates may be summarized as follows:

The weight of evidence indicates a major defensive role in invertebrates for phagocytosis, augmented by relatively nonspecific innate or acquired humoral factors. Preformed substances released into the hemolymph from granular hemocytes seem to play a major role in humoral defenses of Crustacea, and probably other invertebrates as well. Thus the body fluids of many invertebrates contain natural bactericidins, agglutinins, lysins, and occasionally precipitins. Some limited evidence for augmenta-

tion of such defenses by exposure to foreign antigen exists. This evidence is primarily in form of increased titers following experimental inoculation. Specific acquired antibodies (immunoglobulins) have not been demonstrated in invertebrates, but induced antibody-like activity has been demonstrated in a few species. The fundamental difference seems to be in degree of specificity of response, which is significantly higher in the vertebrates. It is obvious that the synergistic action of phagocytes and humoral factors in the invertebrates, as well as in the vertebrates, constitutes the significant defense perimeter—but the degree of specificity of the humoral components is lower in the invertebrates. The master internal defense plan seems to be: foreign protein + humoral factor = recognition of foreignness and phagocytosis.

Cooper (1969c), concluding a very thought-provoking paper, stated: "It seems reasonable to conclude that invertebrates do possess immune systems, although the nature of the mechanisms is decidedly unknown. Reactions may be as numerous as the varied taxonomic groups, as is true of most rigorously studied vertebrates. Invertebrate cellular immunity may be closer to vertebrate reactions and may represent the more primitive responses. In the absence of classic vertebrate-type immunoglobulin in invertebrates, a real dichotomy would be evident in the evolution of immune responses. On the other hand, immunoglobulin precursors may be present."

Certainly the next decade will prove to be an exciting one in the study of invertebrate internal defense systems. The components of classical vertebrate immunity—present as analogues in invertebrates, and probably varying widely among phyla—provide an excellent background against which new findings may be evaluated.

ACKNOWLEDGMENTS

The author would like to acknowledge with thanks the helpful comments of Dr. Harvey Rabin, Institute for Comparative Biology, Zoological Society of San Diego, San Diego, Calif.; Dr. James Stewart, Halifax Laboratory, Fisheries Research Board of Canada, Halifax, N. S.;

and Dr. Marennes Tripp, Department of Biological Sciences, University of Delaware, Newark, Del. Impetus for this review was provided in part by an examination of cellular and humoral responses of shrimps and crabs being conducted with Dr. Ben Sloan, Carson-Newman College, Jefferson City, Tenn. Elizabeth Leonard, Librarian, Tropical Atlantic Biological Laboratory, aided immeasurably in assembling copies of the extensive body of literature on which this review is based.

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CLEANING SYMBIOSIS AMONG CALIFORNIA INSHORE FISHES¹

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ABSTRACT

Cleaning symbiosis among shore fishes was studied during 1968 and 1969 in southern California, with work centered at La Jolla. Three species are habitual cleaners: the señorita, *Oxyjulis californica*; the sharpnose seaperch, *Phanerodon atripes*; and the kelp perch, *Brachyistius frenatus*.

Because of specific differences in habitat, there is little overlap in the cleaning areas of these three species. Except for juvenile sharpnose seaperch, cleaning is of secondary significance to these species, even though it may be of major significance to certain individuals. The tendency to clean varies between individuals. Principal prey of most members of these species are free-living organisms picked from a substrate and from midwater—a mode of feeding that favors adaptations suited to cleaning.

Because it is exceedingly abundant in a variety of habitats, the señorita is the predominant inshore cleaning fish in California. Certain aspects of its cleaning relate to the fact that only a few of the many señoritas present at a given time will clean, and that this activity is not centered around well-defined cleaning stations, as has been reported for certain cleaning fishes elsewhere. Probably because cleaners are difficult to recognize among the many señoritas that do not clean, other fishes generally do not attempt to initiate cleaning; rather, the activity is consistently initiated by the cleaner itself. An infested fish approached by a cleaner generally drifts into an unusual attitude that advertises the temporary existence of the transient cleaning station to other fish in need of service, and these converge on the cleaner. Although señoritas, as a group, clean a number of different fishes, a given individual tends to initiate cleaning with members of just one species.

The fishes cleaned most often are those which are most abundant and, at the same time, are most heavily infested with external parasites. The most numerous ectoparasites are caligid copepods, the most abundant and widespread of which is *Caligus hobsoni*. These particular parasites, along with gnathiid isopod larvae, are the major prey of the cleaning fishes. Cleaning is essentially limited to the external body surface; ectoparasites of the oral and branchial cavities are not ordinarily taken. Cleaning effectively reduces the number of parasites on fishes that are cleaned, and is an important activity for the organisms involved. However, there is no basis for the contention that many good fishing grounds in southern California exist because fishes have congregated in these locations for cleaning.

It has been suggested that many of the better inshore fishing spots are, in fact, cleaning stations (Limbaugh, 1961; Feder, 1966). The contention is that fishes congregate at these locations so that ectoparasites and other deleterious material can be removed from their bodies by resident cleaning organisms. Critics of this hypothesis might well suggest instead that cleaners simply are especially active where fishes are most abundant, or that the cleaners as well as those they clean occur at these locations for

reasons that have nothing to do with cleaning. Regardless of which view is correct in a given situation, one having witnessed fishes crowded around a cleaner, vigorously soliciting its services, can only conclude that this activity is indeed important to the organisms involved.

Cleaning symbiosis has been widely described in the literature (Longley and Hildebrand, 1941; Eibl-Eibesfeldt, 1955; Limbaugh, 1955, 1961; Randall, 1958, 1962; and others) and was reviewed by Feder (1966). Youngbluth (1968) studied activity of the Hawaiian cleaning labrid *Labroides phthiophagus* in some detail, and Losey (1971) analyzed the communicative signals between this same species and the fishes that it cleans. But most other reports on cleaning have been simple treatments based largely on

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incidental observations. In this report, I describe cleaning symbiosis among inshore fishes of southern California and attempt to relate observed activity with the incidence of specific ectoparasites.

Conrad Limbaugh, Scripps Institution of Oceanography, was among the first to report cleaning symbiosis among California fishes. In a study of fishes of the kelp beds, Limbaugh (1955) described cleaning by the señorita, *Oxyjulis californica*, a fish of the family Labridae, and also by several seaperches of the family Embiotocidae: the kelp perch, *Brachyistius frenatus*; the black perch, *Embiotoca jacksoni*; and the pile perch, *Rhacochilus vacca*. Subsequent observers have described cleaning by the rainbow seaperch, *Hypsurus caryi* (Gotshall, 1967); the sharpnose seaperch, *Phanerodon atripes* (Clarke, Flechsig, and Grigg, 1967; Gotshall, 1967; Hobson, 1969a); and the blacksmith, *Chromis punctipinnis* (Turner, Ebert, and Given, 1969).

SPECIES STUDIED

Most of the cleaning observed during this study was performed by the señorita (Figure 1), which by virtue of its great abundance in a variety of habitats is the predominant cleaner inshore. The sharpnose seaperch (Figure 2) was frequently observed cleaning, but its activity is centered in deeper water. The kelp perch (Figure 3) may be an important cleaner in the canopy region of the kelp forests, where it concen-

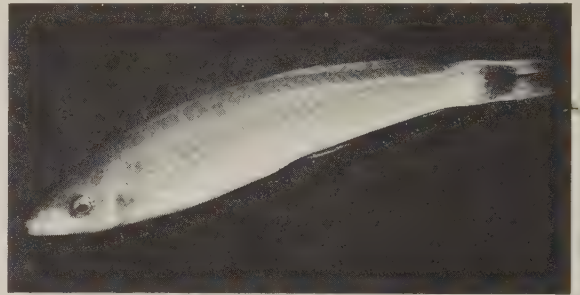


FIGURE 1.—Señorita.

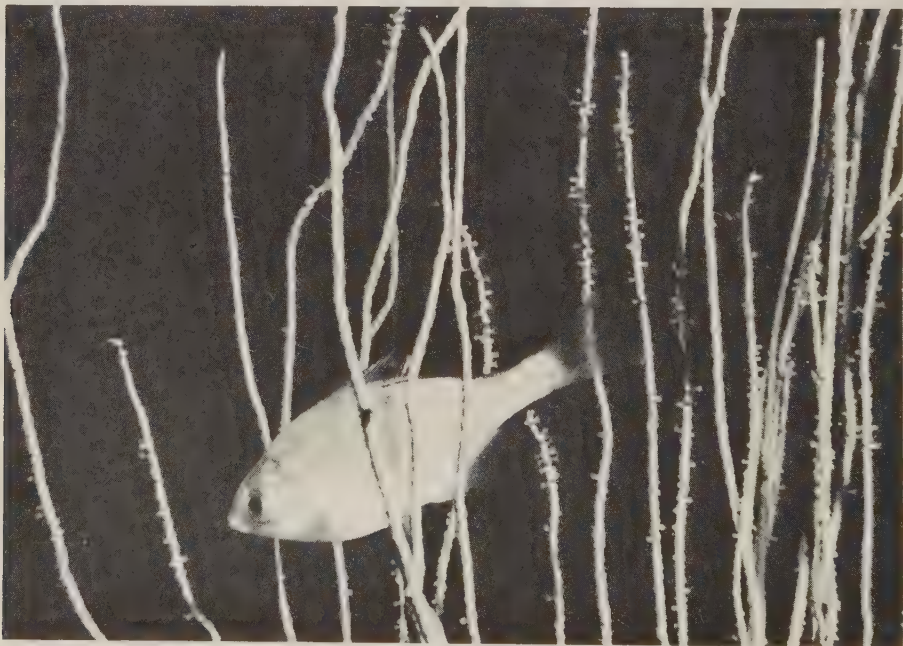


FIGURE 2.—Sharpnose seaperch among branches of a gorgonian.

trates, but this was not determined in this study because observations in the kelp-canopy habitat were infrequent. Nevertheless, observations were sufficient to recognize the kelp perch as a habitual cleaner. The only other fish seen cleaning was the white seaperch, *Phanerodon furcatus*, in which cleaning seemed to be only an occasional incidental activity.

METHODS

During 1968 and 1969, I spent more than 103 hr underwater directly observing cleaning and related activity in California inshore waters. Also contributing to the study are many incidental observations of cleaning made during other work with California fishes between 1961 and 1970.

Supplementing the observations, 421 specimens of 39 species were collected with spear. These represent most of the species common in the study area that exceed a length of 100 mm (all lengths of fishes in this report are standard length). The ectoparasites were collected from

all specimens and will be reported in detail elsewhere in collaboration with R. F. Cressey, U.S. National Museum. These collections also provided the material for descriptions of 11 species of copepods formerly new to science (Cressey, 1969a, 1969b, 1970; J. Ho, California State College, Long Beach, unpublished manuscript). Additional undescribed species may occur among a number of copepods from these collections presently under study by Z. Kabata, Biological Station, Nanaimo, British Columbia.

In addition to a survey of the ectoparasites, gut contents of known cleaning species, including material from 53 señoritas, 29 sharpnose seaperch, and 3 kelp perch, were analyzed.

Many ectoparasites leave their host when it is in difficulty, and some fishes regurgitate their stomach contents under stress. To reduce this loss, all specimens were individually sealed in plastic bags immediately upon capture, and while still underwater.

To acquire detailed data on the cleaning interaction, a number of individuals of cleaning species were kept under surveillance for periods

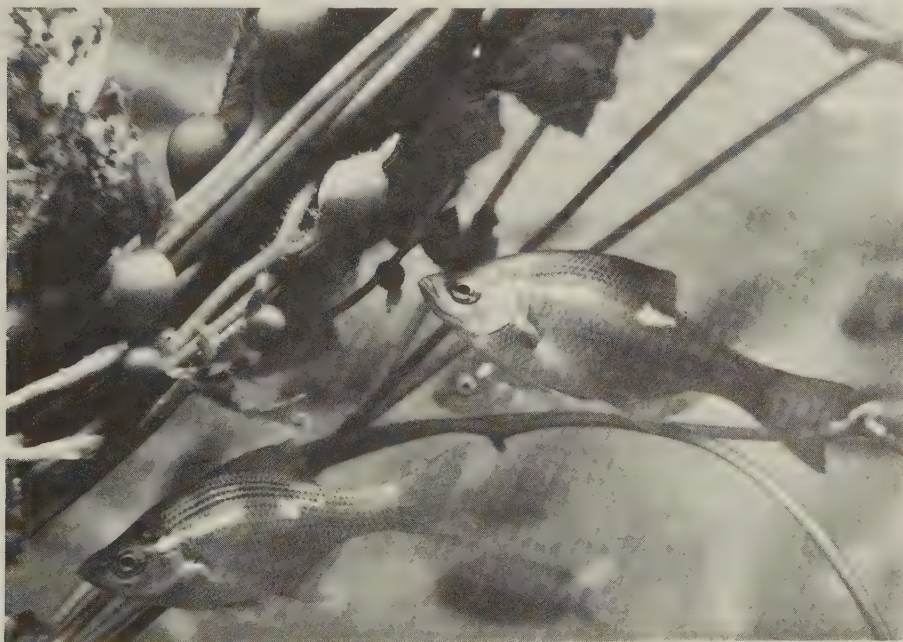


FIGURE 3.—Kelp perch next to giant kelp.

up to 15 min, and a verbal account of their activity was recorded on tape. The attempted standard of 15 min could not be maintained for all these observations because sometimes contact with the fish being watched was lost as the fish swam among vegetation or other fishes. Individuals followed included known cleaners as well as others that had not shown evidence of cleaning. In monitoring the activity of known cleaners, a record was kept of the time during which they showed an apparent cleaning interest in other fish and also the number of cleaning bouts in which they became involved. A cleaning bout is defined here as any cleaning activity involving a discrete group of fishes, whether this group includes one cleaner attending a single fish, or several cleaners attending a cluster of 40 to 50 fish. On various occasions I also recorded the number of times that the cleaner actually "picked" at the body of another fish, and precisely at what point on the body this action was directed.

Study Areas

Observations of cleaning symbiosis were made at many locations throughout southern California, including the Channel Islands, and at the Coronado Islands, Mexico. However, most of the data were collected during concentrated work at La Jolla, Calif. Here, three study sites were established, each including an area of about 100 m², that lie on a line running northwest offshore from La Jolla Point. Moving away from the beach along this line, the first station lies in 3 to 10 m of water about 200 m offshore, the second is in 20 to 25 m of water about 700 m offshore, and the third in 30 to 35 m of water about 1000 m offshore. The sea floor at all three stations is rocky and irregular, with many crevices and caves. Algae are not conspicuous at the two deeper stations, which are similar, but the rocks support a heavy growth of gorgonians. On the other hand, the nearshore region of the 3- to 10-m station is richly carpeted with surfgrass, *Phyllospadix*, and other parts of the inshore station are forested by large kelps, particularly giant kelp, *Macrocystis*, and feather-boa kelp, *Egregia*. However, these large kelps are sparse here

in comparison to some areas nearby to the south and elsewhere in California. Other details of the principal study area will be introduced as they become pertinent.

During all observation periods at the La Jolla stations a record was kept of water temperatures from surface to bottom, horizontal visibility, and surge conditions.

OBSERVATIONS

GENERAL ECOLOGY

Señorita

The señorita, which attains a length of about 250 mm, is one of the most abundant fishes in the inshore waters of southern California, including the Channel Islands. It occurs from the shoreline to depths exceeding 40 m and is recorded from central California south to central Baja California, Mexico (Roedel, 1953). An inhabitant of water over rocky substrates and among sea weeds, the señorita sometimes swims singly, but more often in groups of from a few to many hundreds of individuals. Like other labrids, it is strictly a diurnal fish, taking shelter under cover at night.

Food habits.—The señorita feeds on a variety of benthic organisms from the surface of both algae and rocks. It also feeds heavily in the midwaters, taking small organisms in the plankton, as well as forms that are attached to or encrusted on drifting algal fragments. All this feeding is accomplished in a characteristic picking manner, a mode of feeding well suited to its pointed snout and the several long, curved canine teeth that project forward at the front of each jaw.

To determine the food habits of this fish in the study area, 26 specimens, 110 to 195 mm long, were speared randomly from the population at large. None of these were cleaning when collected. Food items in their stomachs, ranked as percentage of each item in the entire sample, were as follows: bryozoans encrusted on algae, 43%; caprellid amphipods, 32%; fish eggs, 3%; gammarid amphipods, 2.5%; unidentified crustacean fragments, 4%; and pelecypod mollusks,

2.4%. A number of items each made up less than 1% of the sample, including crab fragments, gastropod mollusks, pycnogonids, and a gnathiid isopod larva. Unidentified material constituted 16% of the sample. The gnathiid larva, a single individual from one señorita, was the only evidence of ectoparasites among this material.

Limbaugh (1955) stated that señoritas are omnivorous carnivores which feed on almost any animal material. Quast (1968) concluded that the principal foods of the señorita are small gastropods and crustaceans associated with algae. Because he found no crabs or pistol shrimps in the diet, Quast suggested that bottom feeding is infrequent; however, having seen señoritas frequently picking on the bottom, I believe there must be some other reason why these prey are not taken more often. Size may be a factor, as crabs and pistol shrimps generally are larger and more heavily shelled than most prey of the señorita.

Movements.—Although individual señoritas may range widely over the bottom in a given locality, they seem to operate within a restricted range. Individual fish, when followed, always criss-cross back and forth within a defined area. Twelve individuals, selected randomly from the population at large and kept under surveillance for 11 to 15 min each, showed no evidence of cleaning.

Señoritas are most abundant at the 3- to 10-m station and become progressively fewer with increasing depth offshore. Nevertheless, even at the 30- to 35-m station the species was among the most numerous present. Fluctuations in numbers were often apparent with changes in water temperature. Some of the movement is vertical. When a layer of colder water moved in over the bottom—a frequent phenomenon at the 20- to 25-m station—señoritas were especially abundant up in the water column above the thermal interface. Seasonal and other longer term changes may induce inshore/offshore movements in certain members of the population. The numbers present fall off noticeably when temperatures drop much below 13° C, but at least some señoritas were present no matter what the con-

ditions. These comments on temperature effects are based entirely on casual evaluations of relative abundance under varying conditions.

Sharpnose Seaperch

The sharpnose seaperch is not regarded as a common species (e.g., Limbaugh, 1955), but was relatively abundant during this study over rocky substrates below 20 m in the La Jolla area. It grows to over 200 mm long and is recorded from Bodega Bay, central California (Miller, Gotshall, and Nitsos, 1965), south to the San Benito Islands, Mexico (Roedel, 1953). Most of those observed during this study were juveniles less than about 125 mm long that swam singly or, more often, in small groups of less than 10 individuals. Adults were seen only occasionally but sometimes swam in larger aggregations. All activity observed in these fish occurred during daylight. After dark they hover above the substrate and are alert, but their activity at this time, if any occurs, was not determined.

Food habits.—This seaperch takes a variety of benthic organisms from the surface of rocks, algae, gorgonians, and other benthic substrates. Prey are taken off the bottom in a characteristic picking manner similar to that of the señorita. However, the seaperch's dentition would seem less specialized for picking than that of the señorita; its conic teeth are relatively short and straight, and those at the front of the jaws are not notably longer than those on the sides, nor do they project forward.

To investigate the food habits of this fish in the study area, 13 individuals, 76 to 170 mm long, were speared randomly from the population at large. None of these were cleaning when collected. Food items in their stomachs, ranked as percentage of each item in the entire sample, were as follows: caprellid amphipods, 56%; chitons, 9%; planktonic copepods, 9%; isopods, 8%; limpets, 2%; polychaete mollusks, 1%; and sponges, 1%. Unidentified material made up 14% of the sample. There was no evidence of ectoparasites in this material. One individual had fed heavily and exclusively on planktonic copepods, showing that this fish is not

limited to benthic prey. I have found no references to food habits of this fish in the literature.

Movements.—On the basis of limited observations, these fish do not seem to move around in their habitat as much as señoritas do. Nevertheless, they do show marked inshore/offshore movements that may relate to changing water temperatures. Unlike the ubiquitous señorita, this fish occurred in limited numbers that allowed assessing relative abundance through actual counts. It was never seen at the 3- to 10-m station but was reasonably abundant (10-20 individuals were counted during 15-min periods) on all visits to the 30- to 35-m station. At the 20- to 25-m station its appearance was irregular and closely followed temperature fluctuations. Generally it was rare or absent at the 20- to 25-m station when bottom temperatures rose much above 13° C, and was present (a maximum of 10 was seen during a 20-min period) when the temperature dropped much below this level. As most of the individuals seen were juveniles, a seasonal factor independent of temperature was probably operating here. Nevertheless, short-term temperature changes over the critical range (approximately 12°-14° C at the 20- to 25-m station) were consistently accompanied by the presence or absence of this fish. I emphasize that these assessments of abundance are relative to the numbers of the species regularly present. The señorita was always more abundant than the seaperch at all stations and under all conditions. Thus whereas the seaperch was considered abundant during a period in which 15 individuals were seen, at no time did I find so few señoritas present at any of the three La Jolla stations.

Kelp Perch

The kelp perch was not abundant in the La Jolla study area, where it was seen only at the inshore station. Its distribution is essentially limited to the kelp beds, which were not well developed in the study area at the time of this work. Nevertheless, it is very numerous in California inshore waters that are heavily forested

with kelp. Attaining a maximum length of about 150 mm, the kelp perch is recorded from Vancouver Island, Canada, south to central Baja California, Mexico (Roedel, 1953). The kelp perch occurs near the rocky bottom at the base of giant kelp, as well as adjacent to the rising kelp stipes, but is most abundant just under the kelp canopy, near the water's surface. Typically, this fish occurs in aggregations of a dozen or more, but larger individuals frequently are solitary, especially those near the rocky sea floor. Most of my observations of kelp perch were made outside the La Jolla study area, the majority around the Channel Islands.

Food habits.—This perch feeds in a picking manner, similar to that employed by the señorita and sharpnose seaperch. It preys on a variety of organisms from the surface of the surrounding kelp and also feeds extensively on material suspended in the current. Its pointed snout and small, upturned mouth, together with a number of relatively long, curved canine teeth that project forward at the front of each jaw, are well suited to its mode of feeding. The dentition of this fish is similar to that of the señorita, a fact also noted by Hubbs and Hubbs (1954). I did not sample kelp perch from the population at large for food-habit analysis; all those collected were from known cleaning stations. However, Limbaugh (1955) stated that they feed on small crustaceans, particularly those that occur on giant kelp. Quast (1968), who also reported a predominantly crustacean diet, with a preponderance of amphipods, noted that some mollusks and bryozoans are taken as well.

Movements.—Limited observations indicate that aggregations of kelp perch in the canopy, and close to large rocks, remain relatively stable. Several aggregations that were observed over 2 to 3 months did not change appreciably in location or in numbers of individuals. Data on this point are scanty, however.

At night they hover in the same areas in which they are active in daylight, but their activity at this time, if any occurs, was not determined.

CLEANING ACTIVITY OF THE SEÑORITA

Unlike some other cleaners (see Feder, 1966), señoritas do not establish well-defined stations at which they receive other fishes seeking to be cleaned. Rather, the señoritas, as they move over the local area, approach and clean fishes wherever they encounter them.

Despite their great abundance, only a small segment of the señorita population seems pre-disposed to clean at a given time. The cleaning habit is not limited to any particular stage in their life history: cleaning señoritas have included some of the smallest individuals seen (< 40 mm) as well as some of the largest (> 225 mm). In cleaning material from the bodies of fishes, señoritas employ the same picking technique they use to take small prey from a rock or algal substrate. This mode of feeding, along with their pointed snout and long, forward-projecting canine teeth, are well suited to the cleaning habit.

Individuals that clean are numerous where there are many resident fishes, especially of certain species (as discussed below), but I found no evidence that residents of other areas come to these locations to have parasites removed. Occasionally a migrating species, such as the California yellowtail, *Seriola dorsalis*, will pause to be cleaned while passing through areas where cleaners are active, but this is not the same as a resident of a particular area habitually swimming elsewhere to be cleaned and then returning to its home ground.

Fishes Cleaned by the Señorita

Casual observation alone show that some fish species are cleaned far more often than others, and that many species do not seem to interact with cleaners at all.

To obtain data on this point, a record was kept of the species seen being cleaned by señoritas during 62 observation periods (15 min to 2 hr long) from June 1968 to January 1969. During this period, 392 cleaning bouts were witnessed, 385 of which involved señoritas cleaning one or more individuals of a single species; in only

seven instances were señoritas seen cleaning members of a mixed-species group. The tabulation of species cleaned (Table 1) does not include the mixed-species groups because in the mixed groups it was not determined whether representatives of all species present were actually cleaned. All seven mixed groups included halfmoons, *Medialuna californiensis*, and one or more fish of other species. In four of these, halfmoons were mixed with blacksmiths, in one they were mixed with opaleyes (*Girella nigricans*), in one with rubberlip perch (*Rhacochilus toxotes*), and in one with both rubberlip perch and pile perch. All of these were incidental observations. The compilation does not include data obtained on other occasions when the activity of individual cleaners was recorded for extended periods.

The data clearly indicate that blacksmiths, and to a lesser extent topsmelt (*Atherinops affinis*), predominate as recipients of the señorita's cleaning efforts in the areas where the observations were made. Table 1 is not a definitive list of species cleaned by the señorita; nevertheless, it is evident that many species which co-occur with the señorita are not cleaned. At other times, in addition to all species noted in Table 1, I have seen *Seriola dorsalis* and *Trachurus symmetricus* being cleaned. But the ratio of species listed here generally is consistent with observations made on other occasions and at many different locations.

TABLE 1.—Fishes observed being cleaned by señoritas during 62 observation periods between June 1968 and January 1969 at La Jolla, Calif. (exclusive of seven mixed-species groups).

Species	Number of cleaning bouts	Percent of total bouts observed
Blacksmith, <i>Chromis punctipinnis</i>	231	60
Topsmelt, <i>Atherinops affinis</i>	81	21
Garibaldi, <i>Hypsypops rubicunda</i>	22	6
Halfmoon, <i>Medialuna californiensis</i>	19	5
Señorita, <i>Oxyjulis californica</i>	10	3
Rubberlip perch, <i>Rhacochilus toxotes</i>	8	2
Opaleye, <i>Girella nigricans</i>	5	1
Kelpfish, <i>Heterostichus rostratus</i>	3	1
Black perch, <i>Embiotoca jacksoni</i>	1	1
Pile perch, <i>Rhacochilus vacca</i>	1	1
Sargo, <i>Anisotremus davidsoni</i>	1	1
Blue rockfish, <i>Sebastes mystinus</i>	1	1
Olive rockfish, <i>Sebastes serranoides</i>	1	1

Reports in the literature present a comparable picture. Most published accounts of cleaning by señoritas describe the way blacksmiths cluster around this cleaner to solicit its attentions (Limbaugh, 1955, 1961; Feder, 1966; and others). Limbaugh (1955) observed the following fish being cleaned by señoritas: *Myliobatis californica*, *Stereolepis gigas*, *Paralabrax clathratus*, *Trachurus symmetricus*, *Atherinops affinis*, *Anisotremus davidsoni*, *Hyperprosopon argenteum*, *Rhacochilus vacca*, *Chromis punctipinnis*, *Hypsypops rubicunda*, *Girella nigricans*, *Medialuna californiensis*, and *Mola mola*. Turner et al. (1969) observed the following fish being cleaned by señoritas: *Sebastes* spp., *Atherinops affinis*, *Atherinopsis californiensis*, *Trachurus symmetricus*, *Seriola dorsalis*, *Chromis punctipinnis*, and *Mola mola*. Neither of these reports gives data on the relative frequency with which these different species were cleaned, but it is significant that many of the same species consistently appear in the reports of independent observers, while at the same time many other species that frequent these waters in large numbers are not mentioned. No doubt many species not yet reported are occasionally cleaned by señoritas, but there seems little doubt that a certain few species, the blacksmith in particular, predominate in this activity.

Specific Cleaning Interactions

The fishes cleaned by the señorita vary markedly in their habits and habitat, as well as in their relative numbers. These fishes do not seek out cleaning at a "station" established by the señorita, but rather receive the señorita on their own grounds during the course of their regular activity. Cleaning interactions often proceed differently with one of these species than with another. Some of these variations in cleaning activity are characterized below.

Señorita-blacksmith interactions.—The blacksmith is one of the most abundant fish over rocky substrates in California inshore waters, where it swims in large stationary aggregations in midwater. It feeds largely on zooplankton (Quast, 1968) and attains a length of about 250 mm.

Generally the first sign of an interaction occurs when a señorita swims up alongside a blacksmith in midwater and closely inspects its body. The blacksmith may then immediately stop swimming and, holding its fins motionless and erect, drift into an awkward-appearing posture. Usually the blacksmith is head-down, but sometimes turns on its sides or is tail-down. On some occasions the blacksmith presents a particular part of its body to the inspecting señorita. The señorita swims about this fish, usually pausing briefly to pick at its body. Immediately following the first sign of this activity other blacksmiths converge on the spot, so that very quickly 10 or more crowd around the cleaner (Figure 4). The señorita soon leaves the original blacksmith and may then move on to one of the others. It may also swim slowly away, whereupon the group of blacksmiths follows along, each attempting to position itself in the señorita's path. Although the señorita shows progressively less interest in the blacksmiths, they continue to crowd in its way. Soon the señorita shows no further interest in cleaning, and all but a few blacksmiths leave the group. The remaining few doggedly continue attempting to present themselves to the now-unresponsive cleaner. Eventually, however, these last blacksmiths lose contact with the cleaner as it swims off among the kelp or the many other señoritas in the surrounding water. Once they have lost contact with the cleaner the blacksmiths do not attempt to solicit cleaning from any of the many other señoritas around them.

On only two occasions did I note blacksmiths soliciting cleaning from a señorita that did not seem to have made an initiating gesture. Once the blacksmiths were very small, about 40 mm long, and in the other observation, at a depth of 27 m, little cleaning had been seen and relatively few señoritas were present. However, in both instances the señoritas were known by me on the basis of earlier observations to be individuals that clean. It is possible that the fishes soliciting attention recognized these señoritas as cleaners through some cue not noted by me. Sometimes when a señorita incidentally passes close to a blacksmith, the blacksmith noticeably pauses in its swimming and looks as

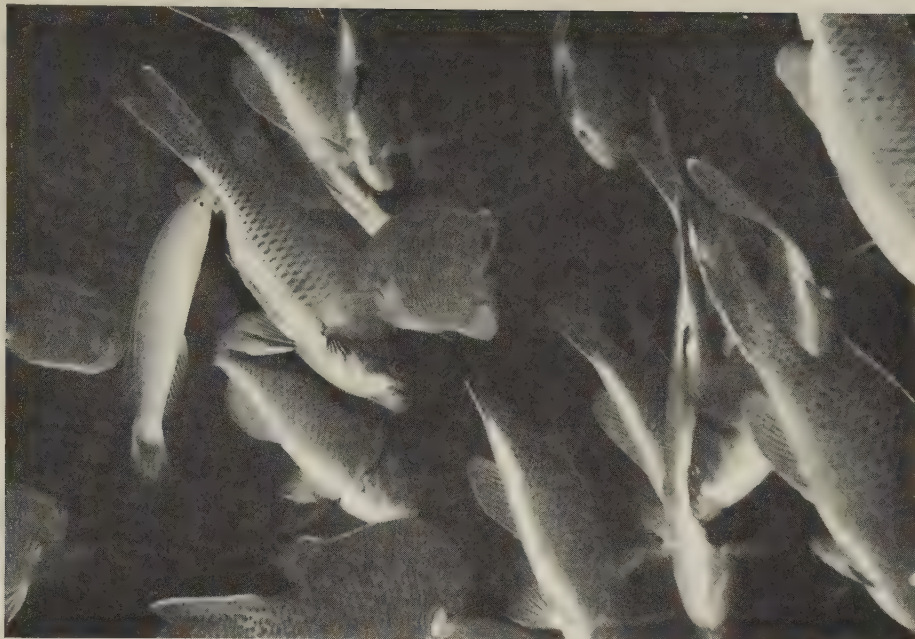


FIGURE 4.—Señorita cleaning the caudal peduncle of one of a group of blacksmiths that hover to solicit service.

though it is beginning to assume a soliciting posture; however, when the señorita swims on past, the blacksmith immediately resumes its original activity. Occasionally members of other species were seen responding similarly to passing señoritas. In most observations of cleaning, my attention was drawn to activity already in progress, so that it was not possible to determine whether cleaner or client had initiated the activity.

Individual señoritas that cleaned blacksmiths during many short-term observations were not seen cleaning any other species. This same situation held true for three individuals, known to have been cleaning blacksmiths, whose activity was monitored in detail on tape for 15 min. When observed for extended periods, señoritas were found to become involved in a succession of separate cleaning bouts. This activity was not restricted to one location but continued at various points over a relatively wide area. Periodically they joined cleaning already underway, or initiated cleaning themselves

at a number of different locations—always with blacksmiths. I have no explanation for the fact that a señorita which becomes unresponsive and leaves one group of blacksmiths that still vigorously solicits its service may soon initiate activity again with another blacksmith.

The three individuals whose cleaning activity was monitored for 15 min joined in a mean of 4 separate bouts (range 2-6). For a mean of 11 min of this time (range 6.75-13.25 min) they showed an apparent cleaning interest in blacksmiths, or were accompanied by blacksmiths with which they had earlier initiated a cleaning interaction. When not thus engaged with blacksmiths, they swam in midwater showing no apparent interest in the fishes around them but occasionally picked at drifting scraps of debris, usually algal fragments. During much of the time that they swam in consort with blacksmiths, they closely inspected these fish and actually picked at their bodies a mean of 26 times (range 14-33). Of these picks, 27% were made at the base of the blacksmith's anal fin, 25% on the

caudal peduncle or caudal fin, 22% at the base of the pectoral fin, 10% somewhere on the body exclusive of a fin-base or head, 8% on the head, 5% at the base of the pelvic fins, and 3% at the base of the dorsal fin.

Clearly, the bases of the fins receive most of the attention from the señoritas. These data are consistent with the many more general observations made on other occasions. At no time during this study were señoritas seen to clean within the oral or branchial cavities of blacksmiths: all cleaning was directed at the body surface.

Señorita-topsmelt interactions.—The topsmelt, which attains a length of about 200 mm, is abundant in many inshore regions of California coastal waters, but its distribution is more spotty than that of the ubiquitous blacksmith. Like the blacksmith, it feeds largely on zooplankton, which it takes while swimming in large schools at the water's surface. Quast (1968) noted the similarity in diet between topsmelt and blacksmiths, and while acknowledging that their feeding areas may overlap, he pointed out that topsmelt normally swim higher in the water column.

In the La Jolla study area, topsmelt are concentrated at the inshore station over extensive fields of surfgrass that grow in 3 to 5 m of water. They are never far from the substrate in this relatively shallow water, even though they swim in large schools at the water's surface. They are more abundant than blacksmiths in this area, and here they predominate in the señorita's cleaning activity.

The cleaning interaction proceeds in much the same way as it does with blacksmiths: the activity is initiated when a señorita swims up to an individual topsmelt and begins to inspect it closely. Immediately other topsmelt converge on this pair to place themselves in the señorita's path, thus soliciting its attention. When presenting themselves motionless before señoritas, topsmelt frequently hover tail-down, in contrast to the head-down posture most often assumed by blacksmiths. I saw señoritas clean only the external body surfaces of topsmelt. In the relatively shallow water where most of this activity was observed, señoritas break off contact with a

group of topsmelt more readily than they do with blacksmiths, as they need only swim down to the substrate below, where the topsmelt seem reluctant to follow.

These shallow areas are frequently swept by surge, and the load of drifting debris in midwater is frequently heavy. In this area cleaning señoritas frequently leave the groups of topsmelt they are attending to inspect an object drifting in the water nearby. Sometimes they take the object into their mouths, sometimes not. Often when taken it is quickly rejected.

Attempts at extended observations on individual señoritas that had been cleaning topsmelt were largely unsuccessful. Too often before the observation had progressed far the señoritas disappeared among the surfgrass or other vegetation carpeting the sea floor in this area. However, two individuals were followed for 10 min each, during which time one entered into four, the other two, separate cleaning bouts. Between cleaning bouts these two swam over a wide area alone in midwater, occasionally picking at drifting debris. On several occasions they picked at benthic algae. Neither individual showed cleaning interest in species other than topsmelt, which was consistent with observations of other señoritas that cleaned topsmelt.

Señorita-garibaldi interactions.—The garibaldi, which attains a length of about 250 mm, is a solitary, highly territorial fish that lives close to the substrate. Especially during the reproductive season, when the males aggressively guard their nests among the rocks, these bright orange pomacentrids normally drive away all other fish that come near. They feed on sessile benthic invertebrates and are abundant at the 3- to 10-m station.

Garibaldis frequently are cleaned by señoritas. Most of the garibaldis seen being cleaned were swimming a meter or so above the bottom; I did not observe cleaners active around the garibaldis guarding nests among the rocks. All of the garibaldis seen being cleaned were solitary, which reflects their territorial nature. The señorita swims up to a garibaldi and closely inspects its body, thus initiating the action. Usually the garibaldi hovers motionless in a normal hori-

zontal attitude, its fins sometimes erect. The señorita may pick at a few places on the garibaldi's body—most often around the caudal region—but usually its attentions are brief, and soon it swims away. With blacksmiths and top-smelt, each cleaning bout is prolonged by the many other individuals that join at the cleaning site to crowd in the señorita's path. Nothing of this sort happens with the solitary garibaldi, which usually makes no attempt to follow the señorita when it leaves, so that each cleaning bout is relatively brief. After leaving one garibaldi, however, often the señorita quickly approaches another. In agreement with their cleaning of blacksmiths and top-smelt, señoritas known to have cleaned garibaldis were subsequently seen cleaning only other members of that same species. This was true during several short-term observations, and also when one individual was followed for 15 min, and a record of its activity was taped; this particular señorita initiated cleaning activity with 26 different garibaldis during the observation period as it swam over an irregular course among the rocks in an area where blacksmiths, top-smelt, and other species also were present. Each cleaning bout lasted a mean of 10 sec (range 7-25 sec), totaling 4 min 15 sec of the 15-min period. In nine of these bouts, the señorita inspected the garibaldi but did not pick at its body. In the other 17 bouts, the señorita picked at the garibaldi's body a total of 42 times, or a mean of about 2.5 times per bout.

All cleaning of garibaldi that I observed was directed at the external body surface.

Señorita-halfmoon interactions.—Halfmoons, which may exceed a length of 250 mm, usually swim high in the water column, frequently in large aggregations, but just as often in small groups or as solitary individuals. They are often abundant among rising stands of giant kelp. Their omnivorous diet, which includes a variety of benthic algae, along with bryozoans, sponges, and crustaceans (Limbaugh, 1955; Quast, 1968), indicates bottom feeding; however, much of this material is taken in midwater as drifting debris.

Considering their large numbers in many southern California coastal areas, halfmoons are

not particularly abundant in the principal study areas. Still, they were frequently seen being cleaned by señoritas during this study. When many halfmoons were present, cleaning by the señorita progressed much as described above for blacksmiths. Yet when just one halfmoon was present, a frequent occurrence, the cleaning bouts were brief like those described above for the garibaldi. At least one halfmoon was present in all the mixed-species groups that I recorded when collecting the data presented in Table 1. I saw señoritas clean only the external body surface of halfmoons.

One señorita, seen cleaning a halfmoon, was kept under surveillance for 12 min before contact was lost. As the observation period began, the señorita picked at the halfmoon once and then moved away, swimming slowly and alone, 2 or 3 m over the substrate. After an uneventful 3 min, the señorita approached a second halfmoon, which promptly hovered in a head-down attitude. For 15 sec the señorita closely inspected this halfmoon and picked at its body three times before swimming away. It then continued on alone for the remaining 8+ min that it was under observation, still swimming slowly over a wide semicircular course 2 or 3 m above the rocks. During this time it passed many different fish without showing interest, but it did not pass another halfmoon. It did pick at three different pieces of floating debris but rejected all three immediately.

Señorita-señorita interactions.—Señoritas themselves are cleaned by other members of their own species. Despite the large numbers of señoritas that usually are present, I saw no groups converging on cleaning individuals, as regularly occurs with blacksmiths, top-smelt, and other abundant species. In most of the señorita's intraspecific cleaning interactions, the cleaner attends just a single individual, which usually hovers motionless in a normal horizontal attitude, except that its fins are erected; sometimes the mouth is open wide and gill covers are distended, but I saw señoritas clean only the external body surface of these fish. There was no indication that señoritas which clean other señoritas also clean other species. I fol-

lowed one individual for 10 min after having seen it clean another señorita. After this initial activity, the individual under surveillance swam over a wide area, showing interest only in other señoritas, even though blacksmiths, top-smelt, and other species were present. Swimming alone, 2 or 3 m over the rocks, it would assume a position alongside another señorita and follow it for a short distance. Usually these other fish showed no interest, but some stopped swimming and erected their fins, whereupon the cleaner picked at their bodies—usually once, but occasionally several times. Between cleaning encounters this señorita passed through a school of very small (< 40 mm) blacksmiths, several of which hovered head-down in its path; however, the cleaner showed no interest in these fish. On two occasions it picked at a piece of drifting debris.

Señorita-kelpfish interactions.—At least one species regularly initiates cleaning bouts with señoritas. Earlier (Hobson, 1965a) I reported observing a kelpfish, *Heterostichus rostratus*, repeatedly soliciting cleaning from unresponsive señoritas. The kelpfish was concealed among benthic algae, which is the typical habitat of this fish. But each time a señorita approached in the water overhead, the kelpfish rose up into the señorita's path, where it hovered motionless, fins erect (Figure 5). A succession of señoritas

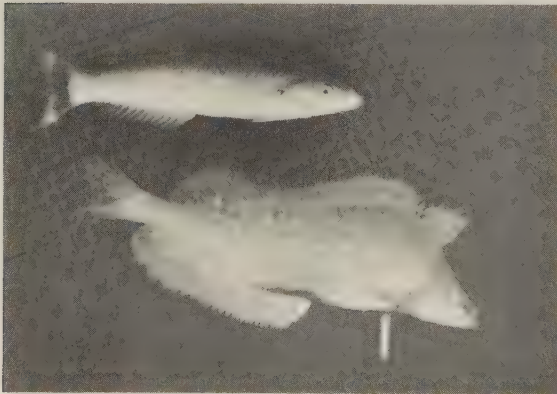


FIGURE 5.—Kelpfish hovering in midwater, fins erect, to solicit cleaning from a passing señorita.

passed by without responding to the kelpfish, and each time the kelpfish returned to the cover below, where it waited until the next approach. Finally a passing señorita paused briefly and picked at the kelpfish's side before continuing on its way. After this brief encounter, the kelpfish did not rise from concealment again even though several more señoritas subsequently passed overhead.

During the present study this sequence of events involving kelpfish and señoritas was witnessed several times at a variety of locations; indeed, every instance of a kelpfish being cleaned followed this pattern—obviously it is a regular pattern in the behavior of the species.

Other interactions.—Observations are too few to recognize distinctive aspects in cleaning interactions involving the many other species that occasionally are cleaned by señoritas. Usually such activity is noted simply as occasional sightings of small clusters of fish, or individual fish, hovering before a señorita. In all such encounters, however, only the external body surface was cleaned.

Notes follow regarding two other species that are cleaned by señoritas.

A single señorita was observed cleaning a pile perch, after which its activity was noted for 15 min. Pile perch are not abundant where these observations were made, and after leaving the first individual, the señorita swam alone in midwater for 14 min. It moved over a wide semicircular course during this time and showed no interest in any of the many fish that it passed, although none were pile perch. It did pick at three small items drifting in midwater. After 14 min it made an abrupt course change and, with slightly accelerated swimming, went directly to a solitary pile perch that was in midwater about 10 m away. The señorita swam about the pile perch, which now hovered head-down, but after a close inspection lasting about 10 sec it moved on without picking at the pile perch's body.

Many of the fishes cleaned by señoritas occasionally start, as if nipped too vigorously. Sometimes such fish dart away, thus terminating the cleaning. Other times they actively turn on the

señorita and drive it away. The rubberlip perch was noted taking the latter course of action perhaps more often than the other species, even considering the relatively few times it was observed being cleaned.

Although the kelpfish is the only species that was observed consistently initiating cleaning from señoritas, individuals of several species do so in at least one situation. This occurs where exceptionally large concentrations of señoritas, sometimes thousands, swim above the rocks. At various times, garibaldi, pile perch, rubberlip perch, olive rockfish, and others were observed hovering in the soliciting posture amid these concentrations (Figure 6) until one of the señoritas approached and cleaned them.

Material Removed from Other Fishes by the Señorita

Food habits of cleaning señoritas.—An obvious question is: What do señoritas remove from the bodies of fishes they clean? Limbaugh (1955) stated that señoritas remove bacteria,

parasitic copepods, and isopods. He was not more specific than this, nor did he present data. Various other cleaners reportedly take not only ectoparasites but also diseased and necrotic tissue (Feder, 1966, and others.)

To determine just what it is that señoritas remove from the bodies of other fishes, I examined the gut contents of 27 specimens, 111 to 175 mm long, that were speared while they were cleaning other fishes. Food items in their guts, ranked as percentage of each item in the entire sample, were as follows: caligid copepods, 39%; gnathiid isopod larvae, 12%; algae with encrusting bryozoans, 10%; caprellid amphipods, 5%; fish scales, 4%; and fragments of nonparasitic crustaceans, 4%. Unidentified material made up 26% of the sample. Of the 27 specimens, ectoparasites occurred among the gut contents of all but two. In most, the ectoparasites predominated. Even though the data are convincing, they do not fully reflect the extent to which cleaning obviously dominated the activity of these particular fish for at least several hours leading up to their capture. This is because the



FIGURE 6.—Garibaldi hovering amid a large assemblage of señoritas.

parasites are so small (1-3 mm long) in comparison with the size of other food items. For example, 471 gnathiid isopod larvae were present in one señorita gut, but being so small they constituted only 35% of the material. On the other hand, a few algal fragments, with encrusting bryozoans, made up 40% of the material in this same specimen. However, only seven individuals contained ectoparasites alone, as compared with 17 that contained both parasites and free-living prey. In all but one of these, the two classes of material were sharply divided in the gut, usually with the free-living material posteriorly in a more advanced stage of digestion. Although most of the ectoparasites in the gut had undergone extensive damage and would not, by themselves, have been identifiable to species, this material usually graded gradually to freshly ingested specimens that were readily identified. This fact, coupled with the circumstance that individual señoritas tend to stay pretty much with a single type of food organism during a given period, greatly aided the task of analyzing this material.

Ectoparasites on the fishes.—To assess the significance of cleaning in removing ectoparasites, one must know what parasites occur on the fishes, as well as the extent of the infestation. Thus the survey of ectoparasites done in conjunction with this work included essentially every fish species exceeding 100 mm long regularly present in the La Jolla study area, as well as every species that was seen being cleaned there. Ectoparasites infesting these fishes include 33 species of copepods, one species of brachiuran, two species of isopods, one species of leech, and one species of monogenetic trematode. Following is a brief summary of the information being compiled on these parasites in collaboration with R. F. Cressey.

Copepods are the predominant ectoparasites on fishes in this area. The 33 species represent seven families: Bomolochidae (6 species), Caligidae (13 species), Dichelesthidae (2 species), Lernaeidae (1 species), Chondracanthidae (5 species), and Lerneopodidae (6 species). The one species of the closely related brachiurans is a member of the family Argulidae. The bomolo-

chids, which were found on 12 species of the fishes sampled, are mobile forms about 2 mm long (all lengths of parasites here and below do not include egg cases) that occurred mostly on the gills of their hosts. The caligids, which infested 29 species of the fishes, are mobile forms, 2 to 4 mm long, that occurred mostly on the external body surface of their hosts, although two species were found only in the oral cavity. The dichelesthids are highly modified forms about 2 mm long that were attached to the gills of two species. The lernaeid is a highly modified form about 5 mm long that was attached to the fins of 12 species. The chondracanthids, which infested five species, are highly modified forms, 3 or 4 mm long, that lived attached in the branchial chamber, including the gills, of their hosts. The lerneopodids, infesting eight of the fish species, are highly modified forms, 2 to 5 mm long, mostly living attached in the branchial and oral cavities, although one individual fish carried several attached to its dorsal fin. Finally, the argulid is a mobile form about 2 mm long that was found on the outer body surface of one species of fish. The fish species hosting representatives of the different copepod and brachiuran families are listed in Table 2, and examples of the six copepod families are illustrated in Figure 7.

Thus a variety of ectoparasitic copepods occur on the fishes, but only caligids were found among the gut contents of the cleaners. Furthermore, although 13 species of caligids (five species of the genus *Caligus* and eight species of the genus *Lepeophtheirus*) occur on fishes in this area, only a relatively few of these are significant as prey of the cleaners, as noted below.

Of the two isopods, one, *Livoneca vulgaris*, a large parasite, about 20 mm long, was found in the branchial chamber of just one species of fish and was not found to be prey of the cleaners. On the other hand, the highly mobile gnathiid larvae (Figure 8), which are about 2 mm long, are a major prey of the cleaners. Only one form of gnathiid was readily recognized, but more than one species may occur among this material. Parasites of the body surface of fishes, the gnathiid larvae were taken on 11 of the fish species sampled, but I suspect that they are actually more widespread and abundant than these data

TABLE 2.—The types of ectoparasites and the fishes they infest, based on a survey of the fishes in the study area. Where more than one species of parasites is included under a heading, the number in parentheses following the name of each fish thereunder indicates how many different species of that type are represented on that fish. For all fish species listed, the number of infested individuals is shown over the number of individuals examined, followed by the range in numbers of individual parasites of that type which were taken from that species of fish.

COPEPODS	COPEPODS—Cont.	ISOPODS
BOMOLOCHIDAE (6 species of 3 genera)	CALIGIDAE—Cont.	CYMOTHOIDAE (1 species)
Fishes infested with members of this family:	<i>Atherinops affinis</i> (1) 13/13: 1-23	Fish infested with this parasite:
<i>Paralabrax clathratus</i> (1) 2/8: 1-2	<i>Pleuronichthys coenosus</i> (1) 5/10: 1-5	<i>Sebastes mystinus</i> 1/9: 1
<i>P. nebulifer</i> (3) 5/11: 1-20	DICHELESTHIIDAE (2 species of 1 genus)	GNATHIID LARVAE (number of species not determined)
<i>Phanerodon atripes</i> (1) 1/13: 1	Fishes infested with parasites of this family:	Fishes infested with these parasites:
<i>Rhacochilus vacca</i> (1) 3/15: 1	<i>Gymnothorax mordax</i> (1) 1/1: 9	<i>Chromis punctipinnis</i> 1/10: 1
<i>Micrometrus minimus</i> (1) 1/7: 1	<i>Paralabrax nebulifer</i> (1) 5/11: 2-70	<i>Hyppyspops rubicunda</i> 4/20: 1-8
<i>Hyppyspops rubicunda</i> (1) 1/20: 1	LERNAEIDAE (1 species)	<i>Pimelometopon pulchrum</i> 3/14: 1-4
<i>Scorpaena guttata</i> (1) 6/14: 2-56	Fishes infested by this parasite:	<i>Sebastes atrovirens</i> 4/16: 1-20
<i>Sebastes mystinus</i> (1) 1/9: 1	<i>Trachurus symmetricus</i> (1) 1/7: 1	<i>S. carnatus</i> 3/11: 1
<i>S. serranoides</i> (1) 2/11: 1-8	<i>Anisotremus davidsoni</i> (1) 1/8: 1	<i>S. chrysomelas</i> 1/11: 2
<i>Oxylebius pictus</i> (1) 1/8: 1	<i>Cheilotrema saturnum</i> (1) 2/16: 1-2	<i>S. constellatus</i> 1/2: 1
<i>Atherinops affinis</i> (1) 2/13: 1-4	<i>Medialuna californiensis</i> (1) 5/13: 2-7	<i>S. serranoides</i> 1/11: 1
<i>Pleuronichthys coenosus</i> (1) 6/10: 1-11	<i>Brachyistius frenatus</i> (1) 1/5: 1	<i>S. serripes</i> 3/15: 1-5
CALIGIDAE (13 species of 2 genera)	<i>Embiotoca jacksoni</i> (1) 2/15: 1-2	<i>Oxylebius pictus</i> 3/8: 1-3
Fishes infested with members of this family:	<i>Hypsurus caryi</i> (1) 2/11: 1-2	<i>Scorpaenichthys marmoratus</i> 3/10: 2-5
<i>Paralabrax clathratus</i> (2) 4/8: 1-5	<i>Phanerodon furcatus</i> (1) 3/12: 1-3	MONOGENETIC TREMATODE (1 species)
<i>P. nebulifer</i> (3) 4/11: 5-27	<i>Rhacochilus toxotes</i> (1) 1/10: 1	Fishes infested with this parasite:
<i>Caulolatilus princeps</i> (1) 1/4: 2	<i>R. vacca</i> (1) 2/15: 1	<i>Medialuna californiensis</i> 6/13: 2-16
<i>Anisotremus davidsoni</i> (1) 1/8: 1	<i>Micrometrus minimus</i> (1) 5/7: 1-6	<i>Girella nigricans</i> 4/10: 1-4
<i>Cheilotrema saturnum</i> (1) 2/16: 1	<i>Atherinops affinis</i> (1) 2/13: 1-4	<i>Rhacochilus toxotes</i> 2/10: 1
<i>Medialuna californiensis</i> (2) 12/13: 1-75	CHONDRACANTHIDAE (5 species of 5 genera)	<i>R. vacca</i> 2/15: 1-18
<i>Girella nigricans</i> (3) 1/10: 1-14	Fishes infested with parasites of this family:	<i>Hyppyspops rubicunda</i> 1/20: 1
<i>Embiotoca jacksoni</i> (2) 2/15: 1-2	<i>Oxyjulis californica</i> (1) 13/38: 1-4	<i>Pimelometopon pulchrum</i> 10/14: 1-26
<i>Phanerodon atripes</i> (1) 5/13: 1-5	<i>Scorpaena guttata</i> (1) 2/14: 1-5	<i>Scorpaena guttata</i> 2/14: 5-8
<i>Rhacochilus toxotes</i> (3) 10/10: 1-10	<i>Scorpaenichthys marmoratus</i> (1) 4/10: 1-4	<i>Sebastes atrovirens</i> 7/16: 1
<i>R. vacca</i> (2) 4/15: 1-11	<i>Heterostichus rostratus</i> (1) 7/13: 1-7	<i>S. constellatus</i> 1/2: 1
<i>Chromis punctipinnis</i> (1) 10/10: 2-39	<i>Pleuronichthys coenosus</i> (1) 7/10: 1-47	<i>S. miniatus</i> 2/5: 1-2
<i>Hyppyspops rubicunda</i> (2) 19/20: 1-144	LERNEOPODIDAE (6 species of 4 genera)	<i>S. serranoides</i> 3/11: 1-26
<i>Pimelometopon pulchrum</i> (3) 13/14: 1-70	Fishes infested by parasites of this family:	<i>S. serripes</i> 2/15: 1-3
<i>Oxyjulis californica</i> (3) 13/38: 1-59	<i>Cheilotrema saturnum</i> (1) 1/16: 1	<i>Heterostichus rostratus</i> 6/13: 1-7
<i>Scorpaena guttata</i> (1) 7/14: 1-14	<i>Girella nigricans</i> (1) 1/10: 1	LEECH (1 species)
<i>Sebastes atrovirens</i> (3) 6/16: 1	<i>Phanerodon atripes</i> (1) 5/13: 1-4	Fishes infested with this parasite:
<i>S. carnatus</i> (2) 3/11: 1	<i>Rhacochilus vacca</i> (1) 1/15: 1	<i>Hyppyspops rubicunda</i> 2/20: 1
<i>S. chrysomelas</i> (1) 1/7: 1	<i>Chromis punctipinnis</i> (1) 1/10: 1	<i>Sebastes serranoides</i> 1/11: 4
<i>S. constellatus</i> (1) 2/2: 1-5	<i>Sebastes atrovirens</i> (1) 1/16: 4	<i>Heterostichus rostratus</i> 2/13: 1-2
<i>S. miniatus</i> (1) 1/5: 2	<i>S. constellatus</i> (1) 1/2: 1	FISHES ON WHICH NO PARASITES WERE FOUND:
<i>S. mystinus</i> (3) 5/9: 1-5	<i>S. miniatus</i> (1) 5/5: 2-8	<i>Xenistius californiensis</i> 0/1
<i>S. paucispinis</i> (1) 3/3: 1	ARGULIDAE (1 species)	<i>Halichoeres semicinctus</i> 0/10
<i>S. serranoides</i> (3) 8/11: 1-10	Fish infested with this parasite:	<i>Coryphopterus nicholsi</i> 0/5
<i>S. serripes</i> (2) 15/15: 1-25	<i>Atherinopsis californiensis</i> 1/1: 2	
<i>Scorpaenichthys marmoratus</i> (2) 8/10: 1-42		
<i>Heterostichus rostratus</i> (1) 2/13: 1-2		

indicate. They are the most mobile of the parasites, and probably many escaped when their host fish was collected. The monogenetic trematode occurred on the outer body surface of 13 species of fishes, and the leech occurred similarly on 3 species. However, neither was found to be taken by the cleaners. The fish species hosting the various isopods and also the trematode and leech are listed in Table 2. Listed also are the three fish-species on which no ectoparasites were found.

The above summary of the survey results gives a general picture of the ectoparasites infesting the fishes that co-occur with the cleaners and might be regarded as a list of the potential prey of the cleaning fishes. The following material considers the parasites that actually are known to be prey.

Ectoparasites in the diet of cleaners relative to ectoparasites on fishes that are cleaned.—Many of the ectoparasites listed above infest

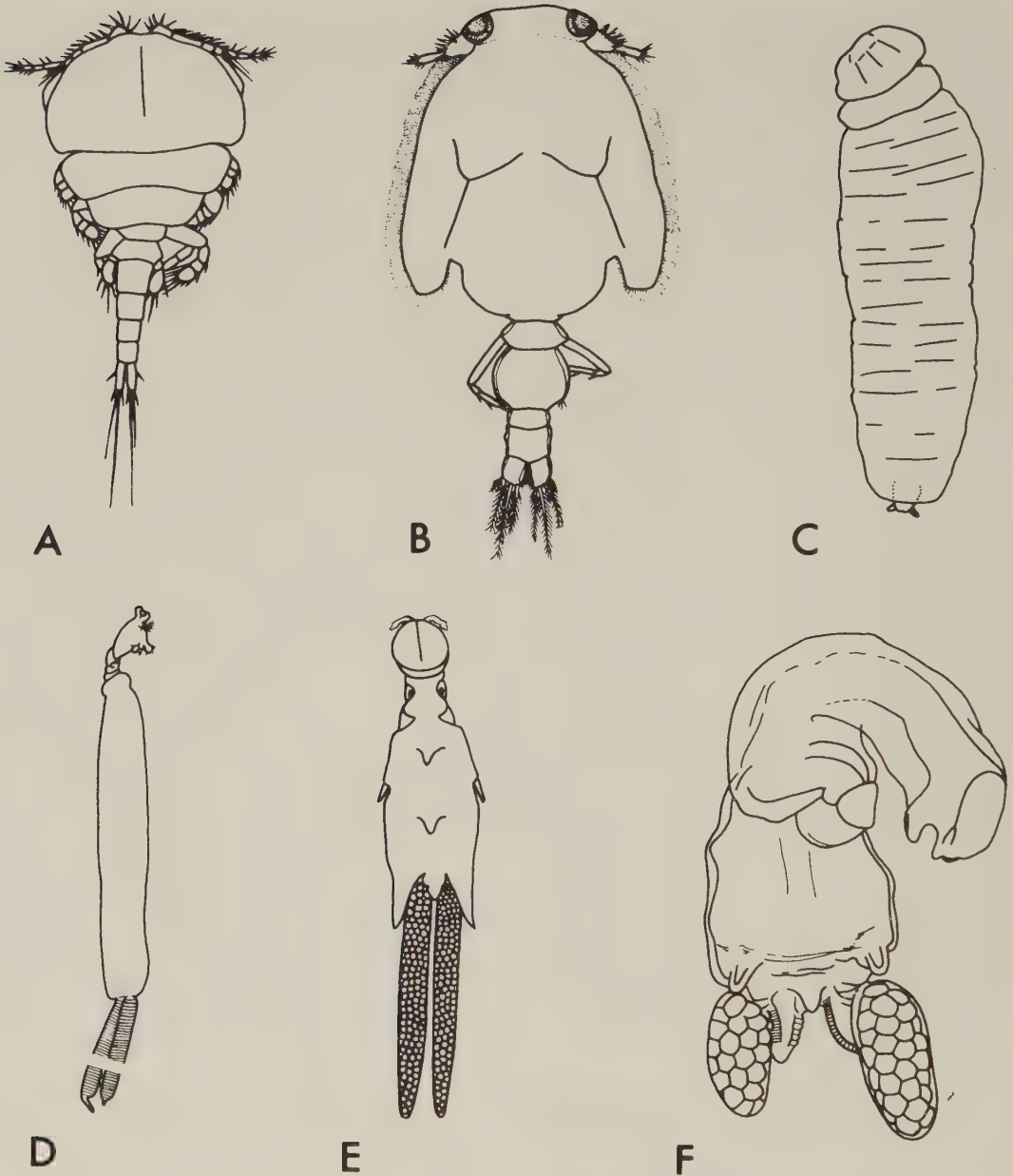


FIGURE 7.—Representatives of the families of ectoparasitic copepods found to infest fishes in the La Jolla area.

- A. Bomolochidae (*Bomolochus longicaudus*, female, after Cressey, 1969b);
- B. Caligidae (*Caligus hobsoni*, male, after Cressey, 1969a);
- C. Dichelethiidae (*Hatschekia pacifica*, female, after Cressey, 1970);
- D. Lerneidae (*Peniculus fissipes*, female, after Wilson, 1917);
- E. Chondracanthidae (*Chondracanthus gracilis*, female, modified after Wilson, 1935);
- F. Lerneopodidae (*Epibranchiella septicauda*, female, after Shiino, 1956).

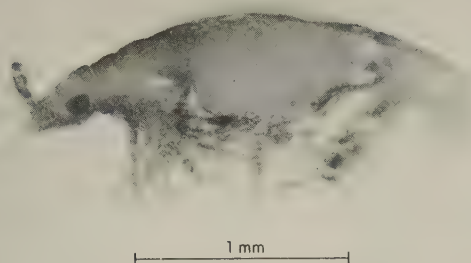


FIGURE 8.—Gnathiid larva from the body surface of the black-and-yellow rockfish, *Sebastes chrysomelas*.

fishes that rarely or never interact with cleaners. In considering the ectoparasites found in the gut of particular señoritas, it would be most meaningful to do so in regard to the ectoparasites known to be hosted by the species of fishes that these particular señoritas were cleaning when collected. Of the 27 cleaning señoritas taken for the gut-content analysis, 15 (56%) were cleaning blacksmiths, 8 (30%) were cleaning topsmelt, 2 (7%) were cleaning garibaldis, and 2 (7%) were cleaning halfmoons. Thus the selection closely parallels the relative frequency with which señoritas were observed cleaning these same species (Table 1) and is a good sample of the fishes that are cleaned by señoritas.

Three species of ectoparasites were collected from 10 blacksmiths, 141 to 199 mm long. Each of these blacksmiths carried from 2 to 39 individuals of the copepod *Caligus hobsoni* on their body surface. One specimen also carried a single gnathiid isopod larva on its body surface, and another the copepod *Clavelloopsis flexicurvica* on a gill arch. All 15 señoritas that were collected as they cleaned blacksmiths contained either *Caligus hobsoni* or gnathiid larvae, but no other ectoparasites: one contained gnathiids alone, seven contained *C. hobsoni* alone, and seven contained both gnathiids and *C. hobsoni*. Up to 256 individuals of *C. hobsoni* and up to 263 gnathiid larvae were counted from among the stomach contents of individual señoritas that had been cleaning blacksmiths.

Three species of ectoparasites were collected from 13 topsmelt, 122 to 212 mm long. These

topsmelt each carried from 1 to 23 specimens of the copepod *Caligus serratus* on their body surface. Two topsmelt also carried the copepod *Parabomolochus constrictus* on their gills, a single parasite on one, four on the other. Two topsmelt also carried the copepod *Peniculus fissipes* embedded in their fins. Six of the eight señoritas that had been cleaning topsmelt when collected had ectoparasites among their gut contents. Five contained only *Caligus serratus*—as many as 73 in each fish. One other contained only 10 gnathiid larvae, a parasite that was not seen on the topsmelt themselves; however, as noted above, I suspect that this parasite is more widespread than our survey data indicate.

Six species of ectoparasites were collected from 20 garibaldis, 184 to 240 mm long. Nineteen garibaldis each carried 1 to 144 *Caligus hobsoni* on their body surface. Thirteen each carried 1 to 4 individuals of an unidentified species of *Lepeophtheirus* on their body surface, and one carried a single *Bomolochus ardeole* in its branchial cavity. In addition, four carried 1 to 8 gnathiid isopod larvae, two carried a single leech, and one carried a single monogenetic trematode, all on their body surface. The two señoritas that were collected as they cleaned garibaldis had preyed mostly on gnathiid larvae, with each containing over 400 of these parasites. In addition, one had consumed six *Caligus hobsoni*, and the other had taken five *Lepeophtheirus* sp.

Four species of ectoparasites were collected from 13 halfmoons, 166 to 295 mm long. Twelve of the 13 halfmoons each carried 1 to 75 *Caligus hobsoni* on their body surface. Each of two also carried a single *Lepeophtheirus* sp. on its body surface, and each of six carried 2 to 7 *Peniculus fissipes* embedded in its fins. In addition, each of six carried from 2 to 16 monogenetic trematodes on its body surface. Of the two señoritas collected as they cleaned halfmoons, each contained only *Caligus hobsoni* in its gut contents, one a single specimen and the other eight.

Significantly, with the exception of the gnathiid larvae in a cleaner of topsmelt, as discussed above, no parasite was found in the cleaner's gut contents that did not occur on the species of fish that was being cleaned by the cleaner

when it was collected. This fact further supports the contention that cleaning tends to be species-specific for a given señorita.

The data clearly show that the parasites most frequently taken by señoritas are certain mobile forms that occur on the body surface of their host. It may be that other parasites on the external body surface are not taken. No leeches or trematodes were found among gut contents, even though these forms are abundant on the garibaldi and halfmoon. Also, the gut contents did not show evidence of the lernaeid *Peniculus fissipes*, an immobile form which partially embeds itself in the skin of its hosts—mostly on the fins. This parasite occurs on topsmelt, garibaldis, and halfmoons among those known to be cleaned by señoritas. However, negative evidence based on the meager gut-content data are weak, especially as the cleaning labrid *Labroides phthiophagus* in Hawaii, which feeds mostly on caligoid copepods, frequently takes lernaeids (Randall, 1958; Youngbluth, 1968). I would expect additional study to show that cleaning señoritas at least occasionally take *P. fissipes*. Nevertheless, several abundant fishes infested by *P. fissipes*, but not found to carry caligids, gnathiids, or other mobile external forms, were not seen being cleaned. For example, the white seaperch is one of the most abundant species at the 3- to 10-m station off La Jolla and yet was never seen being cleaned. Twelve specimens of this fish were examined, and the only ectoparasites found were one to four *P. fissipes* on three individuals. Similarly, the only parasite found on 11 rainbow seaperch, an abundant species in the study areas that was not seen being cleaned, was a single *P. fissipes* on one individual and two on another.

However, not all fishes whose external body surfaces are heavily infested by mobile forms were observed being cleaned. The sheephead, *Pimelometopon pulchrum*, is a case in point. *Caligus hobsoni* occurs on this fish, but only infrequently—a single specimen of this copepod was taken from each of 2 of the 14 sheepheads that were examined. However, the sheephead is heavily infested by two species of *Lepeophtheirus*, a genus of copepods that is closely related to *Caligus*. Up to 70 *L. parvus* were taken from

the body surface of a single sheephead, and this fish has not yet been seen being cleaned. Furthermore, up to 4 gnathiid larvae, which cleaners take from other fish, were found on 3 of the sheephead. Similarly, the treefish, *Sebastes serripes*, which is heavily infested with caligids, has not yet been seen being cleaned. The treefish is not known to carry *C. hobsoni*, but 13 of 15 specimens examined carried up to 12 *Lepeophtheirus longipes* on their body surface, and 3 carried up to 5 gnathiid larvae. The significance of these exceptions to what seems a valid generalization has not been determined. Perhaps it is significant that these two species of fish are not heavily infested by copepods of the genus *Caligus*, as are the more frequently cleaned fishes.

The many parasites that infest the oral and branchial cavities might seem to be potential prey for cleaners, but I found no evidence that these parasites are taken by señoritas.

The principal ectoparasites on the body surface of the two most frequently cleaned fishes, the blacksmith and the topsmelt, are the copepods *Caligus hobsoni* and *C. serratus*, respectively, which are very similar to one another morphologically. With just one exception among the fishes surveyed (discussed below), *C. serratus* seems to be restricted to topsmelt. On the other hand, *C. hobsoni* occurs on a wide variety of species and is also the principal form on garibaldis and halfmoons. Interestingly, a list of the fishes hosting this parasite, ranked by incidence (Table 3), looks much like the ranking of fishes that were observed being cleaned by the señorita (Table 1).

The importance of cleaning in reducing the incidence of ectoparasites on fishes.—Certainly cleaners remove many ectoparasites from the bodies of certain fishes—the numbers in their diet attest to this fact. But does cleaning in fact appreciably reduce the level of infestation on these fishes, or do other parasites quickly replace those that are removed by the cleaners? Although this question is difficult to answer, some insight is provided by observations on the garibaldi. When guarding eggs on their nests during the reproductive season, male garibaldis be-

come especially intolerant of the presence of other fish species. Clarke (1970) recorded the number of times garibaldis, in defence of their territory, attacked fish of various other species at different times of the year. He found that when males were guarding eggs their attacks on señoritas increased elevenfold. Not surprisingly, I saw no cleaning of garibaldis that were guarding eggs. At other times of the year male garibaldis do not guard their territory as vigorously against members of other species and are frequently seen being cleaned. A series of these males were collected both in and out of the reproductive season, and the numbers of ectoparasites they carried were assessed. Seven individuals (mean length 228 mm) sampled as they guarded their eggs carried a mean of 67 *Caligus hobsoni* (range 20-144), 2.5 *Lepeophtheirus* sp., 1.4 gnathiid isopod larvae, and 0.2 monogenetic trematodes. These counts contrast strikingly with those from six males (mean length 219 mm) sampled outside the reproductive season, which carried a mean of only 4.8 *C. hobsoni* (range 0-13), 1 *Lepeophtheirus* sp., 0.8 gnathiid larvae, and no monogenetic trematodes. These findings suggest that males which are guarding eggs become heavily infested with *C. hobsoni* when they do not allow cleaners to approach them, a conclusion strengthened by the fact that over this same period the relative numbers of this same parasite were not noted to change on other in-

festated fishes. The samples included too few of the other parasites to make a meaningful comparison. It remains a question why *Lepeophtheirus* sp. and the gnathiid larvae did not show a pattern of occurrence similar to that of *C. hobsoni*, as both of these parasites are known to be prey of the cleaners. In any event, these data add to the evidence which indicates that *C. hobsoni* is the primary prey of cleaning señoritas in the study areas.

Ectoparasites on Señoritas

Señoritas that were closely observed as they cleaned other fishes often were noted to have caligid copepods on their bodies. One señorita, about 120 mm long, was host to an estimated 100 of these parasites concentrated especially along the dorsal-fin base. These observations were significant because during the survey for ectoparasites, most señoritas taken from the population at large were free of external forms, although many carried a chondracanthid copepod on their gills.

Twenty señoritas, 102 to 190 mm long, were sampled from among those giving no indication of being cleaners. Eight of these carried 1 or more of the chondracanthids on their gills, but only 2, or 10%, had parasites on their external body surfaces: one of these carried 10 specimens of *Caligus hobsoni* and 1 specimen of

TABLE 3.—Hosts of *Caligus hobsoni*.

Species	Specimens examined	Specimens hosting <i>C. hobsoni</i>	Number of <i>C. hobsoni</i> on each infested fish mean (range)	Percent occurrence
Blacksmith, <i>Chromis punctipinnis</i>	10	10	10.6(2-39)	100
Topsmelt, <i>Atherinops affinis</i>	13	13	110(3-23)	100
Garibaldi, <i>Hyppypops rubicunda</i>	20	19	31.2(1-144)	95
Halfmoon, <i>Medialuna californiensis</i>	13	12	19.8(1-75)	92
Opaleye, <i>Girella nigricans</i>	10	8	5.4(1-14)	80
Olive rockfish, <i>Sebastes serranoides</i>	11	5	2.6(1-4)	45
Blue rockfish, <i>Sebastes mystinus</i>	9	4	1	44
Sharpnose seaperch, <i>Phanerodon atripes</i>	13	5	2(1-5)	38
Señorita, <i>Oxyjulis californica</i>	36	9	11(1-59)	24
Sheephead, <i>Pimelometopon pulchrum</i>	14	2	1	14
Rubberlip perch, <i>Rhacochilus toxotes</i>	10	1	1	10
Cabezon, <i>Scorpaenichthys marmoratus</i>	10	1	1	10
Gopher rockfish, <i>Sebastes carnatus</i>	11	1	1	9
Pile perch, <i>Rhacochilus vacca</i>	15	1	1	7
Kelp rockfish, <i>Sebastes atrovirens</i>	16	1	1	6

¹ *Atherinops affinis* does not carry *C. hobsoni*, but rather is the sole host (with one exception, see text) of the very similar *C. serratus*.

Lepeophtheirus sp.; the other señorita carried a single *Lepeophtheirus* sp. Comparative data were obtained by examining 16 señoritas, 114 to 160 mm long, that had been cleaning. Of these, 11, or nearly 70%, carried copepod parasites on their external body surfaces: 6 carried from 1 to 59 *Caligus hobsoni*, 4 carried from 1 to 9 *C. serratus*, and 1 carried 3 *Lepeophtheirus* sp.

Significantly, those señoritas carrying *Caligus hobsoni* all had been cleaning blacksmiths, those carrying *C. serratus* had been cleaning topsmelt, and the one carrying *Lepeophtheirus* sp. had been cleaning a garibaldi. Thus the ectoparasites found on cleaning señoritas were in all instances forms that also infest the species which that particular señorita had been cleaning. The occurrence of *C. serratus* is especially interesting, because these señoritas are the only fish other than topsmelt found so far to carry this parasite.

Alerted to the phenomenon, I inspected the bodies of many señoritas that incidentally passed by during various phases of the work underwater. Ectoparasites were evident on some, but only on a small minority of the population. That the vast majority are not infested by such parasites accounts for the observation, noted above, that señoritas do not crowd around cleaners that initiate activity in their midst, as do blacksmiths, topsmelt, halfmoons, and others.

On the basis of these data, and on the general cleaning picture that has developed, I believe that at least most of the señoritas infested with caligid copepods are cleaners. Presumably they acquire these parasites while intimately associated with the former hosts during cleaning. That a given cleaner is found to carry parasites similar to those on the fish it has been attending, but no others, is further evidence that cleaning by individual señoritas tends to be species-specific.

Environmental Factors That Influence Cleaning

Temperature.—As noted above, the numbers of señoritas present at the 20- to 25-m station fluctuated in an apparent response to water temperature, with the critical level at about 12° to 13° C. Less cleaning occurred at lower temperatures (Figure 9), which would be ex-

pected with fewer señoritas present. Nevertheless, even considering the smaller numbers, the señoritas present at lower temperatures seem less active than those present at higher temperatures. The effect was striking on one occasion at 25 m when, with an influx of warm water, the temperature rose suddenly from 11° to 14.5° C. No change was noted in the numbers of señoritas present over this short period of time, but where no cleaning had been seen during a 20-min survey immediately before, shortly after the temperature rise six different groups of fishes being cleaned were in view simultaneously.

Turbidity.—When the water is turbid because of plankton or suspended sediment, there is noticeably less cleaning activity than when the water is clear. The fishes are generally more wary, and remain closer to cover when visibility is reduced.

Surge.—When there is a strong surge, a frequent occurrence, especially in water less than 10 m deep, there is far less cleaning activity than when the water is still.

Day-night.—The señorita, a strictly diurnal species that takes shelter under cover at night, does not clean after dark.

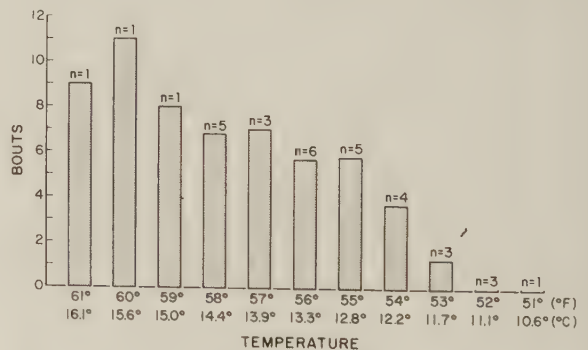


FIGURE 9.—Number of señorita cleaning bouts seen during each of 33 observation periods, 15-25 min long, at different water temperatures in an area 25 m deep at La Jolla. Periods during which temperature fluctuated were not considered. n = number of observation periods at that temperature; where $n > 1$, value given is the mean.

CLEANING ACTIVITY OF THE SHARPNOSE SEAPERCH

Unlike señoritas, which clean as adults as well as juveniles, all of the sharpnose seaperch that I observed cleaning were juveniles less than about 125 mm long. Occasionally noncleaning seaperch swim in groups of 15 or more, but those seen cleaning were always solitary, or in groups of two or three. In agreement with señoritas, cleaning seaperch do not establish well-defined cleaning stations, but instead may clean other fish at any point as they move from place to place. I found no evidence that fishes which are residents of other areas come to where seaperch are located for cleaning; rather, cleaning seaperch occur where resident fishes are numerous. As is true of señoritas, seaperch use the same picking technique to clean material from the bodies of other fish that they use to take small organisms from a benthic substrate. Clearly bottom-picking can be preadaptive to cleaning. Cleaning by seaperch, as by señoritas, usually occurs within 3 m of the substrate. However, there is little overlap in the cleaning areas of the two species: generally seaperch clean at greater depths and/or in colder water than señoritas, where limited observations indicate they may predominate as cleaners even when señoritas are more abundant. Data illustrating this distribution of cleaning activity at a point in time were obtained at the 20- to 25-m and 30- to 35-m locations off La Jolla, where the two species co-occur

(Table 4). Despite the fact that señoritas were observed to be far more numerous than perch throughout the depth range of this study (3-50 m), seaperch performed almost all the cleaning observed at the 30- to 35-m location, where cleaning by the much more abundant señorita was limited to a few isolated instances.

A measure of the incidence of cleaning individuals within the population of juvenile sharpnose seaperch was obtained during 39 observation periods at the 20- to 25-m and 30- to 35-m locations at La Jolla. These observations, totaling more than 26 hr, were made from September 1968 to February 1969. During this period, 201 juvenile seaperch were seen, of which 105, over 52%, were cleaning other fishes. Thus it appears that at least most sharpnose seaperch are cleaners when they are juveniles, whereas only a small minority of the señorita population seem to be cleaners.

Fishes Cleaned by the Sharpnose Seaperch

Because sharpnose seaperch were observed only at depths below 20 m, substantially less data are available on their cleaning activity than on that of señoritas. Of the 105 seaperch observed cleaning during the 39 observation periods reported above, all but one were cleaning blacksmiths; the lone exception was cleaning a solitary blue rockfish, *Sebastes mystinus*. On two other occasions, I saw sharpnose seaperch cleaning rubberlip perch, but otherwise the only fish seen being cleaned have been blacksmiths (Figure 10). Undoubtedly additional observations, especially in other areas, would expand this list. I observed señoritas cleaning in many different areas, but my observations of cleaning seaperch are limited to La Jolla. Clarke et al. (1967) saw a sharpnose seaperch cleaning a rockfish at 150 m off La Jolla, and Gotshall (1967) reported what he believed to be this species cleaning *Mola mola* off Monterey. Yet no matter how many different species the seaperch may in fact clean, there seems no doubt that blacksmiths are prime recipients in southern California, at least in depths shallower than 35 m.

TABLE 4.—Number of bouts in which sharpnose seaperch and señoritas, respectively, were seen cleaning other fishes during 15-min observation periods at the 20- to 25-m and 30- to 35-m locations off La Jolla. Two observation periods, one at each location, and never more than 45 min apart, were made on each of the dates indicated.

Date	Number of cleaning bouts observed			
	20- to 25-m location		30- to 35-m location	
	Seaperch	Señorita	Seaperch	Señorita
22 Nov.	2	13	17	0
27 Nov.	0	7	1	0
9 Jan.	2	5	9	0
15 Jan.	2	4	8	0
3 Feb.	2	12	9	2
Total	8	41	44	2

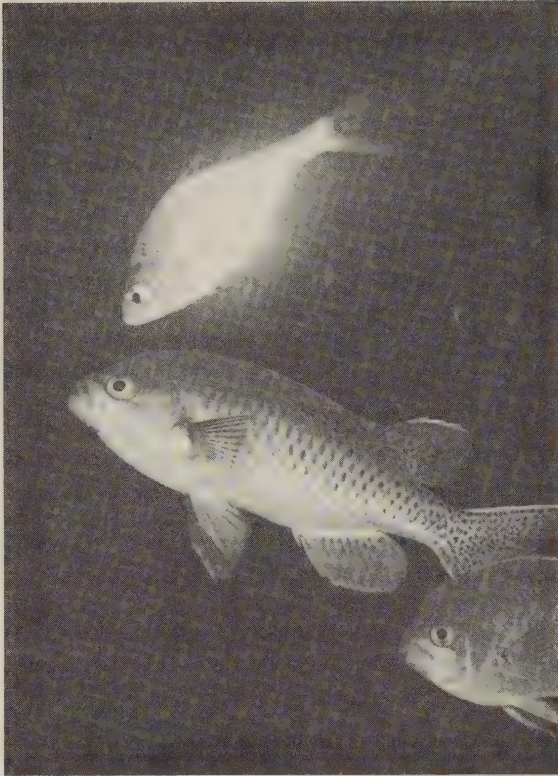


FIGURE 10.—Sharpnose seaperch inspecting a blacksmith, which hovers to solicit cleaning.

Specific Cleaning Interactions—Seaperch-Blacksmith

The limited observations on cleaning by sharpnose seaperch provide details only on interactions with blacksmiths. As nearly as could be seen, when sharpnose seaperch clean blacksmiths the activity proceeds much as it does when blacksmiths are cleaned by señoritas, as described above. However, the observations were too few to determine whether or not cleaning activity is consistently initiated by the cleaner. Several times blacksmiths hovered in their typical head-down posture before seemingly unresponsive seaperch, but perhaps the seaperch had earlier made some initial gesture. Whenever it could be determined, the seaperch initiated the cleaning.

Some details were obtained at the 20- to 25-m location at La Jolla, where two seaperch, known

to have been cleaning blacksmiths, were each kept under surveillance for 15 min, while their activity was monitored on tape. Both swam on irregular courses among the rocks but remained within an area encompassing about 15 to 20 m². During this time one entered into 4, the other 5, separate cleaning bouts, averaging 2.6 (range 0.5-7.5) and 1.8 (range 0.75-3.5) min long, respectively, all with blacksmiths. The cleaner initiated the activity in each instance, but immediately thereafter a number of other blacksmiths converged on the spot. Most of the cleaning bouts continued after the original blacksmith had left the group, and a succession of others arrived and departed before the bout ended. Although usually they hovered head-down before the cleaners, the blacksmiths nevertheless assumed a wide variety of attitudes. During much of the time they swam with the blacksmiths, the two seaperch under surveillance closely inspected the blacksmith's bodies and actually picked at them 18 and 14 times, respectively. Most of the cleaning was directed at the fin bases, particularly the caudal. While in company with the blacksmiths, one of the seaperch broke away from the group and swam to look closely at the dorsal fin of a blue rockfish. However, no cleaning occurred: the blue rockfish swam away as though uninterested in cleaning and the seaperch returned to the blacksmiths. When not in company with the blacksmiths, the two seaperch swam alone 1 or 2 m over the substrate. One descended to the bottom twice and picked at gorgonians: five times on the first descent, once on the second.

Once a blacksmith was seen obviously attempting to present its caudal fin to a seaperch, without success in enticing the seaperch to clean. Close inspection did not reveal parasites, but part of the fin was torn away and shredded flesh was exposed. Apparently this blacksmith was presenting a point of irritation to the cleaner, which in this instance was an injury, not a parasite. Some cleaners, for example, *Abudefduf troschelii*, which picks molting skin from the Galápagos marine iguana (Hobson, 1969b), will clean dead or injured tissue, but at least on this occasion the seaperch showed no interest.

Material Removed from Other Fish by the Sharpnose Seaperch

To determine the food of cleaning seaperch, I examined the gut contents of 16 specimens, 74 to 122 mm long, that were speared as they cleaned blacksmiths. Food items in their guts, ranked as percentage of each item in the entire sample, were as follows: caligid copepods, 68%; caprellid amphipods, 16%; gnathiid isopod larvae, 9%; algae, 1%; and unidentified items, 6%. Thus ectoparasitic caligids and gnathiids made up 77% of the material. All 16 specimens contained ectoparasites; in fact, ectoparasites constituted the vast bulk of the material in all but one individual, which had fed more heavily on caprellids. As with señoritas, when an appreciable amount of free-living material was present, it was usually sharply divided from the ectoparasites and more digested to the rear in the digestive tract. All the identifiable caligid copepods among this material were *Caligus hobsoni*, which is consistent with what is known of ectoparasites on blacksmiths, the species cleaned by these seaperch, and indicates feeding habits similar to those of the cleaning señorita, presented above.

Incidental Cleaning by a Close Relative

Although sharpnose seaperch were not seen in water less than 20 m deep, the white seaperch, a very similar species, is frequently abundant there. The white seaperch was probably the most numerous of the embiotocids during most of the observations made at the 3- to 10-m location off La Jolla. Underwater the white seaperch and the sharpnose seaperch are nearly identical, but can be distinguished by the dusky bordered caudal fin of the former and the black-tipped pelvics and more pointed snout of the latter.

White seaperch are especially abundant in groups of 10 or more close to surfgrass in 3 or 4 m of water off La Jolla. Typically they hover head-down; in this attitude they are not soliciting cleaning but rather are intently regarding the surface of the vegetation, at which they pick occasionally. Tiny organisms that live on the surfgrass are prey of these fish: five white sea-

perch, 80 or 81 mm long, speared in this habitat, were filled with (showing percent of total volume) caprellid amphipods (80%), gammarid amphipods (5%), isopods (2%), fragments of algae with encrusting bryozoans (10%), and unidentified crustacean parts (3%). Quast (1968) found that specimens from a kelp bed had fed mostly on small bottom-dwelling crustaceans, polychaetes and bivalves, as well as kelp fragments, some of which were heavily encrusted with bryozoans. Thus the bottom-picking feeding habits of the white seaperch are very similar to the noncleaning habits of the sharpnose seaperch.

On one occasion, I saw a white seaperch swim 1 or 2 m above the surfgrass in company with a lone blacksmith, which hovered head-down in the manner typical of one that desired to be cleaned. The white perch picked at the blacksmith's body several times, but the bout was brief, and the perch soon joined a group of 8 to 10 others of its own kind near the surfgrass below. This seaperch, which proved to be 79 mm long, was speared, and its gut contents included 58 caprellid amphipods, a single gammarid amphipod, one small isopod, plant fragments with encrusted bryozoans, and some unidentified nonparasitic crustacean remains. No ectoparasites were found; its food was similar to that of the other white seaperch reported above. On another occasion I saw a white seaperch cleaning several blacksmiths over a sandy bottom in 12 m of water, but this individual was not collected. Probably the observed cleaning was no more than a brief incidental activity for these fish. At no other time did I see any indication of cleaning by this species, but perhaps the activity is more frequent under appropriate conditions.

CLEANING ACTIVITY OF THE KELP PERCH

Because the kelp perch is not abundant in the La Jolla study area, where larger kelps are sparse, most observations of cleaning by this fish were made incidentally during other projects in areas heavily forested with kelp. However, these other projects generally were centered on the sea floor, whereas kelp perch concentrate

above in the midwater and canopy regions. Nevertheless, observations of cleaning were sufficiently frequent to recognize this species as a habitual cleaner, though probably less so than either the señoritas or the juvenile sharpnose seaperch. In taking material from the bodies of other fishes, the kelp perch uses the same picking technique that it employs to pick items from an algal substrate, or that are adrift in midwater. Its pointed snout and dentition, which is similar to that of the señorita, as described above, are well suited to cleaning.

Insofar as an aggregation of kelp perch tends to remain in one location, these fish can perhaps be regarded as maintaining a station at which other fishes are cleaned. But I saw no indication that more than one or a few members of a given aggregation clean, or that other fishes come to these locations from any distance for cleaning. In fact, I saw only blacksmiths and other kelp perch being cleaned by this fish. In the one observation of intraspecific cleaning, a single kelp perch swam among others of its aggregation, intently inspecting their bodies. Usually the subject of this attention moved away, whereupon the cleaner moved to another fish. A few responded to the cleaner by erecting their fins and hovering immobile in a head-down posture, and these were cleaned. Occasionally a fish being cleaned suddenly darted away as if the cleaner had been too vigorous in its attentions. All blacksmiths being cleaned were solitary individuals that hovered in head-down soliciting fashion close to an aggregation of kelp perch. Whether or not one of the perch had earlier made an initiating overture was never determined. Never more than one or two of the perch in the aggregations were seen cleaning these blacksmiths. Occasionally a cleaner would closely follow a halfmoon or kelp bass that incidentally passed close by, but I saw no evidence that these fish were interested in the perch, and no cleaning occurred.

Three kelp perch, 94 to 99 mm long, one of which had been cleaning a blacksmith, were collected from an aggregation hovering near a stand of feather-boa kelp. The gut contents of the individual known to have cleaned the blacksmiths contained (showing the percent of total

volume): gnathiid isopod larvae (50%), non-parasitic isopods (5%), gammarid amphipods (5%), caprellid amphipods (20%), and unidentified material (20%). Neither of the two that were not known to have cleaned contained evidence of ectoparasites: one was full of caprellid amphipods (90%) and unidentified material (10%), whereas the other had nothing in its digestive tract except a few unidentified fragments posteriorly.

Limbaugh (1955) reported kelp perch cleaning kelp bass, opaleyes, garibaldi, blacksmiths, and walleye surfperch (*Hyperprosopon argenteum*).

DISCUSSION

Various cleaning fishes remove a wide variety of deleterious material from the bodies of the animals they service. In addition to ectoparasites, this material includes diseased, injured, or necrotic tissue, fungi, and unwanted food particles (Feder, 1966; Hobson, 1968, 1969b; and others). However, the discussion below considers cleaning only as the removal of ectoparasites, because my data indicate that these are the only items taken in significant amounts from California fishes by the cleaners considered in this report.

INCIDENTAL VS. HABITUAL CLEANING

Cleaning is widespread among small-mouthed marine fishes that characteristically pick minute organisms from the substrate (Hobson, 1968). Included are species of the families Chaetodontidae, Pomacentridae, Labridae, Embiotocidae, Blenniidae, and others. Morphological and behavioral characteristics suited to their way of life have preadapted many species of these families for the cleaning habit. Probably some such fishes pick ectoparasites only incidentally during routine foraging when under certain conditions the body of an adjacent fish, infested with ectoparasites, becomes accessible as just another feeding substrate. The relative tendency of a given species to clean likely is influenced by both short-term and long-term environmental changes. Such changes may be expected to alter

interspecific relations, by affecting not only the relative availability of various prey organisms and the incidence of various ectoparasites, but also the species composition of the interacting fishes themselves.

In California the white seaperch likely is one of those species that cleans only occasionally as an incidental adjunct to regular foraging. Several other California species reported by Limbaugh (1955) and Gotshall (1967) clean, including black perch, pile perch, and rainbow seaperch, but they have not been seen doing so by me. The report of cleaning by the blacksmith (Turner et al. 1969) remains an anomaly, as this fish does not fit the pattern of a bottom-picking predator described above. However, it may be significant that many of those substrate-picking predators which clean most frequently are species that also feed on material adrift in mid-water, as do the señorita, sharpnose seaperch, and kelp perch. Thus this mode of feeding too, including the taking of plankton, may, in some species, favor adaptations that are suited to cleaning. Fishes that are adapted to both substrate-picking and plankton-picking may possess adaptations especially well suited to cleaning.

Probably many species of fishes clean incidentally on isolated occasions, but relatively few are habitual cleaners. And even the habitual cleaners vary greatly in the degree to which they are specialized for this habit. Species of the Indo-Pacific labrid genus *Labroides* are highly specialized cleaners that feed almost exclusively on ectoparasitic crustaceans (Randall, 1958; Youngbluth, 1968). These fishes possess many specific morphological and behavioral specializations that are adapted to this way of life (Feder, 1966; Losey, 1971). However, only a small minority of cleaners are so highly specialized; most are but part-time practitioners of the cleaning habit, with much of their food being derived from other sources.

That some cleaners depend on ectoparasites for prey, whereas others can subsist equally well on food from other sources, has led to classifying various species as either obligate or facultative cleaners (e.g., Youngbluth, 1968). The señorita, sharpnose seaperch, and kelp perch may well resist being so classified because their cleaning

seems to be characteristic not so much of a species as of just certain individuals. At least at a given time, most señoritas do not clean, whereas some seem to be facultative cleaners, and a few might even be obligate cleaners. Juvenile sharpnose seaperch follow a similar pattern, but with a relatively higher incidence of individuals that clean. Limited data can only suggest that the status of the kelp perch may be similar.

CLEANING INITIATED BY THE SEÑORITA

Usually there seem to be fishes present that need cleaning, as shown when a señorita identifies itself as a cleaner by initiating action with, say, a blacksmith or a topsmelt, and immediately is converged upon by many other fish that crowd in its way seeking attention. That such fishes generally wait for a señorita to begin the cleaning, rather than attempting to initiate activity themselves with one of the many señoritas present, likely reflects a low probability of success if they make the first move. If, as it seems, the vast majority of señoritas are not cleaners, or at least not currently predisposed to clean, then random efforts to solicit service would not seem adaptive.

This situation contrasts with that of the Hawaiian wrasse *Labroides phthirophagus*, of which all individuals seem to be obligate cleaners (Youngbluth, 1968), and which is not nearly as abundant on Hawaiian reefs as the señorita is in California. In centering their activity around well-defined stations, the distinctive *L. phthirophagus* can be recognized readily by others that need cleaning. Thus, not surprisingly, cleaning encounters that involve *L. phthirophagus* are regularly initiated by fishes seeking cleaning (Losey, 1971).

We have seen that under certain circumstances various fishes initiate cleaning encounters with señoritas. Some fishes successfully do so by hovering amid unusually dense concentrations of señoritas, but the overtures of such fish are not directed at individuals; rather, they are broadcast to the assemblage at large. The success of this tactic presumably follows the probability that an individual predisposed to clean occurs among such a large number of señoritas.

Kelpfish regularly solicit cleaning from individual señoritas, but the situation is exceptional. Because kelpfish rise into midwater for cleaning, it appears that they do not receive satisfactory service in their regular habitat amid benthic vegetation. In their usual surroundings, where they are extremely difficult to discern, the cryptic kelpfish may be relatively inaccessible to cleaning señoritas. One can see why a fish thus handicapped might be required to initiate needed cleaning itself. The number of unsuccessful attempts experienced by kelpfish before a señorita was finally induced to clean them underscores the existing problem of locating a cleaning individual.

SPECIES-SPECIFIC CLEANING

Because the cleaning señorita initiates most of its activity, it has the opportunity to select its clients, and the data indicate that a species-specific choice is exercised. That individual cleaners tend to limit their selection to members of only one species may be related to the fact that they initiate cleaning on the home ground of the fishes they serve, when these fishes are engaged in some of their regular activity. As each of these clients has distinctive habits, a señorita approaching to clean a fish of one species faces a somewhat different situation than a señorita approaching to clean a fish of another species. The distinctions often are subtle, but may be significant enough to account for a given señorita's tendency to seek out members of only one species.

Again we can draw a contrast with the cleaning behavior of *Labroides phthiophagus*, individuals of which receive members of many different species at well-defined cleaning stations (Randall, 1958; Youngbluth, 1968). Probably such nonspecific cleaning is characteristic of cleaners whose activity is confined to these established locations. Fishes that visit such cleaning stations enter the cleaner's own territory, and frequently join a mixed-species group that hovers in wait for service. In tending these fishes on its home ground, the cleaner is receiving them on its own terms, so to speak, so that the situations surrounding cleaning bouts with

all of the different species are essentially the same.

Cleaning by the señorita may not be species-specific on those few occasions when the cleaning activity is initiated by the fish in need of such service, for example by the kelpfish, as described above. Although they show some difficulty locating a receptive señorita, kelpfish nevertheless seem far more successful at doing so than one would expect if indeed they are required to find one that will clean only kelpfish. Thus, although individual señoritas seem to be species-specific when they themselves initiate cleaning, they may be considerably less so, and perhaps even non-specific, when the other fish makes the initial overture. There are no data on this point, however.

The extent to which these considerations apply to juvenile sharpnose seaperch and kelp perch cannot be ascertained because data are lacking.

SIGNIFICANCE OF POSTURES ASSUMED BY FISHES THAT SOLICIT CLEANING

When members of an assemblage of fishes like blacksmiths or topsmelt converge on a cleaning site that has developed in their midst, probably their attention was initially alerted by the unnatural-appearing posture assumed by the individual first approached by the cleaner. Usually this posture does not seem to be assumed purposefully, but rather results when the fish, having ceased swimming and immobilizing its fins, passively drifts out of its regular attitude (Hobson, 1965b). The posture thus assumed varies, especially between species, where perhaps differing centers of gravity are determining factors. Thus the blacksmith usually hovers head-down, whereas the topsmelt is more often tail-down. Sometimes an unnatural-appearing posture is actively assumed when the fish attempts to present to the cleaner a certain part of its body, presumably that part carrying an irritation. By virtue of their unusual appearance, these postures in cleaning interactions serve to draw attention to the fish that is cleaned. It does not seem necessary that any particular posture be assumed, only that it look out of the

ordinary. Reports are widespread (see Feder, 1966) of cleaning recipients assuming these unnatural-appearing postures.

Attention-getting postures assumed by fishes being cleaned probably occurred incidentally during the early development of cleaning symbiosis, when fishes hovering to be cleaned quite naturally stopped moving and passively drifted out of their regular attitudes. As the various cleaning relations evolved, apparently this obvious cue subsequently assumed a different role as a signal in different situations. Generally these postures are suggested to be signals between the recipient of cleaning and the cleaner, indicating a readiness to be cleaned. Quite likely this is the primary signal-function in activity involving such cleaners as *Labroides phthirophagus*, where all members of the species are cleaners and where activity is centered around cleaning stations that are well known to other fishes in the area. In this situation a fish in need of cleaning should be reasonably successful in advertising its condition by assuming the characteristic soliciting posture before a fish recognizable as a cleaner. Losey (1971) showed that various fishes regularly employ this tactic to induce *L. phthirophagus* to clean them. Observations in the Gulf of California demonstrated that the cleaning station itself has played a role in establishing the soliciting posture as a cue. There I have seen the goatfish *Mulloidichthys dentatus* hovering head-down at cleaning stations of the butterflyfish *Heniochus nigrostris*, when the resident cleaner was itself temporarily absent. Losey (1971) observed similar behavior among Hawaiian fishes. In such a situation the hovering posture probably alerts the cleaner to fishes that have arrived for cleaning.

However, in cleaning activity involving the señorita, the soliciting posture usually is assumed only after cleaning has been initiated by the cleaner. The problem of recognizing an individual that will clean among the vast majority of señoritas that do not clean, coupled with the absence of well-defined cleaning stations, would reduce the adaptiveness of the client's soliciting posture as a cue to initiate cleaning. Probably the most effective way for a fish to obtain needed cleaning in this situation is to wait until a cleaner

has identified itself by initiating activity with some fish in the area. Once this has occurred, one can see the value of the posture, when assumed by the first fish to be approached, as a cue in alerting other fish that need cleaning to the presence of a cleaner. In effect, then, the fish assuming the soliciting posture advertises the temporary existence of the transient cleaning "station" to other potential recipients of cleaning. Well-defined cleaning stations like those of *Labroides phthirophagus* do not need this sort of advertisement, as their locations are well known to the fishes that visit them. Nor is it necessary that cleaning individuals of *L. phthirophagus* be pointed out, as all members of that distinctive species are cleaners. Despite this, it is probable that fishes hovering to be cleaned at a *Labroides* station themselves create a visual cue that tends to attract other fishes.

There may also be a maladaptive aspect to the postures assumed by fishes that solicit cleaning. In hovering at an unnatural angle, fins immobile and erect, a fish may enhance its chances of being cleaned, but it would also seem likely to draw the attention of predators and to handicap itself in evading attack. Perhaps such an increased vulnerability accounts at least in part for the sharp decline in cleaning that occurs with reduced visibility, when predators can approach closer undetected. Increased vulnerability may also account for the observation reported earlier (Hobson, 1965c), where pomadasyids in the Gulf of California abruptly broke away from cleaners when a predator approached.

THE POSSIBILITY THAT FISHES BEING CLEANED EXPERIENCE STRESS

Being prodded and picked over by an animal of another species would seem to require a difficult adjustment for a fish. It may well be that fishes experience stress under this circumstance, even when the behavior is well established. Certainly observations have shown that this experience can be uncomfortable, judging from how often fishes being cleaned suddenly bolt forward, and swim away, apparently having been nipped too vigorously by the attending cleaner. Sometimes too, a fish approached by a cleaner clearly

experiences conflicting responses, one moment tolerating or even soliciting the cleaner's attentions, and the next moment chasing it away on each approach. Such ambivalent behavior was especially evident in rubberlip perch. Losey (1971) noted that *Labroides phthiophagus* in Hawaii is sometimes attacked by fishes that it attempts to clean, and suggested that this may occur when the cleaning is painful to the host fish.

The color changes shown by many fishes being cleaned (Randall, 1958; and others) may in fact be manifestations of stress. It is well known that many fishes experience color changes in response to stress. Earlier (Hobson, 1965a) I discussed the striking color change of the goatfish *Mulloidichthys dentatus* when it solicits cleaning in the Gulf of California, and pointed out that this fish shows the same color change in other situations that are obviously stressful. Such color changes have been regarded as signals between the fishes being cleaned and the cleaners, (e.g., Feder, 1966), functioning in the cleaning interaction much like the soliciting attitudes discussed above. As with the attitudes, any role such color changes may now have assumed as a signal probably evolved from an incidental by-product of early cleaning. I have no data on this point relating to the California species, as such color changes are not especially evident in fishes that were observed being cleaned there.

ARE CLEANERS IMMUNE FROM PREDATION?

Reportedly some cleaners are immune from predation because of the service they provide the predators (Feder, 1966; and others). Limbaugh's (1961) belief that the señorita enjoys such immunity is based on observations of this labrid entering the open mouth of kelp bass to clean and on not finding it among the stomach contents of predators during a food-habit study. However, Quast (1968) found señoritas in the stomachs of kelp bass, and H. Geoffrey Moser, U.S. National Marine Fisheries Service (unpublished data), found señoritas in stomachs of the bocaccio, *Sebastes paucispinis*, and the starry rockfish, *S. constellatus*.

I doubt that cleaners enjoy immunity in the sense that predators, recognizing them as benefactors, actively avoid preying on them. Cleaners may recognize those predators which are not at that time intent on feeding and may restrict their cleaning to such individuals. A predator that assumes a soliciting posture may effectively advertise this situation, and no doubt other cues exist. Such mechanisms would reduce the chance of cleaners placing themselves in vulnerable situations while cleaning. In addition, cleaners probably are not as vulnerable while cleaning large predators as might be expected simply because cues characteristic of feeding situations are not present. In associating themselves so intimately with predators, cleaning fishes show behavior that is so unlike that of prey that predators probably do not regard them as food. However, even if such factors do reduce the danger that might seem inherent in the cleaning act, I doubt that their cleaning role affords these fishes any security from being eaten in non-cleaning situations.

PARASITES AS PREY OF THE CALIFORNIA CLEANERS

It is hardly surprising that the fishes which are cleaned most frequently in California are those which are the most abundant and at the same time carry the most ectoparasites. Thus the blacksmith, topsmelt, halfmoon, and garibaldi are the fishes cleaned most frequently, and the survey of ectoparasites showed them to be among the most heavily parasitized. The vast majority of ectoparasites on these particular fishes are mobile forms, mostly caligid copepods and gnathiid isopod larvae, that occur on the body surface of their hosts. That these same parasites were found to make up the diet of the cleaners attending these fishes is consistent with the observation that only the exteriors of fishes were seen being cleaned during this study.

Although the forms infesting the external body surface are the most numerous ectoparasites on the fishes available to the California cleaners, many other types were found to infest the oral and branchial cavities. One might question why these other parasites do not seem to be

taken, especially as Limbaugh (1955, 1961) reported señoritas entering the mouth of the kelp bass and cleaning beneath the gill covers of the garibaldi. Furthermore, such behavior has been widely reported for some other cleaners, such as species of *Labroides* (Eibl-Eibesfeldt, 1955; Randall, 1958; and others), and some echeneids are known to habitually feed on copepods from the branchial cavities of sharks (Cressey and Lachner, 1970). Nevertheless, any such activity by señoritas must be relatively rare. In discussing this situation I limit my remarks to the señorita, because data are presently insufficient to determine whether the same may apply to the sharpnose seaperch and kelp perch.

Señoritas would not be expected to take parasites from the oral or branchial cavities as often as species of *Labroides* or echeneids if for no other reason than they simply are too large relative to most of the fishes they clean. Whereas species of *Labroides* or the echeneids are small enough to enter the oral and branchial cavities of most of the fishes they service, the señorita is nearly as large, and sometimes even larger, than most of its clients. Significantly, Limbaugh observed señoritas cleaning within the oral and branchial cavities of kelp bass and garibaldis, both of which are relatively large species. Most of the señorita's cleaning is directed toward smaller species, like the blacksmith and the topsmelt.

The specialized techniques that would be required to prey on the parasites of the oral and branchial cavities would probably pose another problem to the señorita. In its regular habit of taking parasites from the external surfaces of fishes, the cleaning señorita concentrates on just a few forms that not only are numerous on many of the most abundant fishes, but also are not too dissimilar from free-living prey of the species. Sometimes these external forms also occur in the branchial cavity, and some similar forms, e.g., bomolochids (Figure 7), habitually occur there and in the oral cavity. But the majority of parasites characteristic of the branchial and oral cavities are aberrant forms, e.g., dichelesthiids, chondracanthids, and lerneopodids (Figure 7), and these are unlike anything else encountered by the señorita. No one

type predominates; rather, they occur in a wide variety of forms, none widespread among the different species of fishes, and none especially abundant (except on an occasional individual fish). Thus a cleaner probably could not subsist on one type alone but would have to master a repertoire of specialized techniques in order to exploit enough of these varied forms to make it worthwhile. And before access is gained to the site of infestation, a much more refined cleaner-host interaction must have evolved than is necessary when parasites are simply cleaned from the external body surface. No such relation would evolve unless the cleaner acquired the precise manipulations necessary to pick attached parasites off the gills without damaging the delicate gill membranes. Obviously the cleaning relation would not be adaptive if such damage occurred. In short, to feed habitually on parasites from the oral and branchial cavities would seem to require a higher degree of specialization than has been demonstrated by the señorita. It seems unlikely that such specialization would develop as long as the more abundant and readily available forms on the body surfaces continue to satisfy the cleaning needs of the species. Certainly judging from the way blacksmiths, topsmelt, and other fishes vigorously compete to have their external parasites removed, it would seem that there is little immediate chance of these parasites falling into short supply.

CLEANING SYMBIOSIS AND THE DISTRIBUTION OF SHORE FISHES

In his often-cited report on cleaning symbiosis, Limbaugh (1961: 48) stated:

In my opinion it is the presence of the señorita and kelp perch that brings the deep-water coastal and pelagic fishes inshore to the edge of the kelp beds on the California coast. Most concentrations of reef fishes may similarly be understood to be cleaning stations. Cleaning stations would therefore account for the existence of such well-known California sport-fishing grounds as the rocky points of Santa Catalina Island, the area around the sunken ship *Valiant* off the shore of Catalina, the La Jolla kelp beds and submarine canyon and the Coronado Islands.

Presumably this conclusion was intuitive, as no

data were presented. In his review of cleaning symbiosis, Feder (1966: 368), basing his conclusion on Limbaugh's work, similarly stated: "In all probability, many good fishing grounds are such primarily because they are cleaning stations." I believe that this contention is unfounded. *Señoritas* are the major cleaners in California inshore waters, so that if cleaning symbiosis does account for most concentrations of reef fishes in this region, as Limbaugh suggested, then *señoritas* would be the cleaner largely responsible. Cleaning occurs wherever *señoritas* are concentrated but clearly is not a major activity of the population, even though it may be so for a relatively few individuals. In any event, it seems safe to conclude that cleaning is not among the major factors determining the distribution of *señoritas*. And if cleaning does not determine the distribution of *señoritas* themselves, it seems unlikely that it would determine the distribution of other species.

Undoubtedly many factors contribute to creating situations that draw concentrations of fishes to certain locations. Where a number of different species have similar requirements, assemblages will develop where conditions satisfying these requirements are optimum. The presence of these fishes increases the complexity of the environment, thus creating situations that support still other species, and so on. Often it is apparent that certain features are especially significant as a basis for these concentrations. Consider, for example, the rocky points that Limbaugh included in his list of "well-known California sport-fishing grounds." The flora and fauna of these locations are generally rich, a fact probably related to such local features as converging currents that frequently produce upwelling and nutrient-rich waters. Plankton is commonly abundant here, along with plankton-feeding fishes like the blacksmith. *Señoritas* and other species frequently are numerous here too, but the main attraction seems to be a generally rich food supply rather than available cleaning. Similarly it is unrealistic to attribute concentrations of fishes around sunken ships to cleaning activity. Where a wreck has settled on an open expanse it becomes a haven for fishes that require a nearby structure for cover or a

spatial reference point. Obviously such fishes will center themselves here, because the surrounding featureless substrate does not meet their requirements. I am describing a well-known phenomenon, one that is the rationale behind constructing artificial fishing reefs. Thus food and a suitable substrate often appear to be key features in a habitat that supports large numbers of fishes. Of course to cite just one or the other would be an oversimplification, as requirements in both must be satisfied, along with many other perhaps more subtle needs. The various species assembled at such locations interact in a variety of ways; cleaning symbiosis is one such interaction, and undoubtedly an important one, but hardly the prime reason for them being there.

CHANGES IN HABITS WITH TIME

Uncertainty remains regarding changes in habits with time. The picture of activity developed in this report was derived directly by observing activity and also indirectly by examining both digestive-tract contents and the specific ectoparasites that infest the various fishes. But these methods only define situations that exist over a relatively brief span of time. Data on individual activity over longer periods are needed. Certainly habits of individuals change with time, but how much change and over how much time? The fact that material in the digestive tracts frequently occurs in sharply delimited homologous blocks indicates that these fishes often feed heavily or even exclusively on one particular type of prey, and then abruptly shift to something else. Are habits such as a relative tendency to clean and to clean members of just one species immutable characteristics of individuals, or have the observations described in this report simply defined temporary situations that the various individuals just happened to be experiencing at the time they were singled out for study? It is possible that all *señoritas* clean at one time or another, though not all at once, and only a few at a time. It is also possible that a *señorita*, which tends to clean members of just one species during a given period of cleaning, may select members of another species during

a subsequent period of cleaning. Despite these questions, the conclusions drawn in this report are dependent only on an accurate assessment of the immediate situation, so that their validity is not affected by whether or not the habits of individuals under study remain basically unchanged over time.

A NOTE ON INDIVIDUAL VS. SPECIES HABITS

Information on variations in feeding behavior among individual fish under natural conditions is difficult to acquire. Typically a given behavior is described as a species characteristic, and the extent to which this behavior varies among the different members of the species is unknown. Observations of cleaning by the señorita demonstrate that different individuals in a population may react differently to a given situation. Unquestionably this phenomenon extends beyond cleaning behavior to other facets of the animal's activity. If, as is probable, some of the characteristics of individual fish result from early imprinting, then different members of the same population could be expected to react differently in certain situations throughout life. In any event, it seems unquestionable that the behavior of an individual is considerably more limited than that descriptive of its species, or even its own population.

CONCLUSIONS

1. Three inshore species of fishes in southern California are habitual cleaners: the señorita, the sharpnose seaperch, and the kelp perch. A number of other species clean occasionally as an incidental adjunct to their regular feeding.

2. The señorita may clean throughout its postlarval life, whereas cleaning by the sharpnose seaperch is an activity largely of juveniles. The life-history period during which kelp perch clean has not been defined.

3. Cleaning is of secondary significance to these species, although it may be of major significance to certain individuals. Only a few of the many señoritas present at a given time clean,

and the same seems to be true of the kelp perch. The incidence of cleaners is much higher among juvenile sharpnose seaperch, but the adults of this species do not seem to clean regularly. The major food of all three species is free-living organisms which they pick from a substrate and midwater.

4. There is little overlap between the cleaning areas of the three species. The señorita is the major cleaner in southern California inshore waters by virtue of its great abundance in a variety of rocky habitats. However, the kelp perch may be the predominant cleaner in the canopy region of the kelp beds, where the species concentrates, and the sharpnose seaperch is the predominant cleaner where it occurs at depths below about 20 to 30 m and/or water under 12° or 13° C, even though the señorita may be more abundant.

5. The señorita and sharpnose seaperch do not establish well-defined stations at which they receive other fishes seeking to be cleaned—a situation frequently described for other cleaner fishes. Rather, as they move from place to place, individuals of these species approach and clean other fishes in various different locations.

6. Cleaning activity by these species is essentially limited to removing ectoparasites from the external body surfaces of fishes. They do not ordinarily take parasites of the oral and branchial cavities. The dentition of the señorita and kelp perch, which is similar and which includes a number of long, curved canines that project forward at the front of each jaw, seems especially suited to pick ectoparasites.

7. The major prey taken by these fishes through cleaning are caligid copepods and gnathiid isopod larvae. The species of parasite taken most often by the señorita and sharpnose seaperch is *Caligus hobsoni*.

8. Some species of fishes are cleaned far more often than others, and many species that co-occur with these cleaners are not cleaned at all. The fishes most frequently cleaned are those which at the same time are most abundant and most heavily infested with ectoparasites. The most numerous ectoparasites on these fishes are caligid copepods, the most abundant of which is *C. hobsoni*.

9. Cleaning effectively reduces the number of ectoparasites that infest the external body surfaces of fishes that interact with the cleaners.

10. At any given time, many individuals of the more frequently cleaned species are in need of cleaning. Nevertheless, in activity involving the señorita, infested fishes do not ordinarily attempt to initiate cleaning but instead wait for cleaning to be initiated by the cleaner. Because the vast majority of señoritas are not cleaners, or at least are not currently predisposed to clean, random efforts to solicit cleaning from señoritas in the population at large would not be adaptive.

11. When initiating its cleaning activity, a given individual señorita tends to approach and clean members of only a single species of fish.

12. Because the vast majority of señoritas are not currently predisposed to clean and because there are no well-defined cleaning stations, the unnatural-appearing posture assumed by a fish approached by a cleaner is an important cue in advertising the location of available cleaning to other fish in need of this service.

13. Fishes being cleaned probably experience some degree of stress. The color changes exhibited by some fishes when being cleaned are essentially manifestations of this stress; secondarily, they may have assumed a signal-function in certain cleaning interactions.

14. While intimately associated with the fishes they clean, señoritas frequently become infested themselves by the same parasites they are attempting to remove from these other fishes.

15. Cleaning activity is sharply curtailed when visibility is reduced by turbid water or when there is strong water movement, such as a heavy surge.

16. Cleaning activity among these fishes is a diurnal phenomenon. There is no evidence that it continues after dark.

17. Any so-called "immunity" from predation that a cleaner may enjoy probably relates (1) to an ability to recognize predators that are not intent on feeding and to limit cleaning to such individuals, and (2) to the fact that behavior exhibited by a cleaner servicing a predator is so unlike that of prey that the predator does not regard the cleaner as food. However, their role as cleaners probably does not afford these fish any security

from being eaten during noncleaning situations.

18. Cleaners are widespread among small-mouthed marine fishes that characteristically pick tiny organisms from a substrate. This mode of feeding, especially when combined with the capacity to pick tiny prey that are adrift in mid-water, preadapts fishes to the cleaning habit.

19. There is no basis for the contention that many of the good fishing grounds in southern California are such because fishes have congregated to be cleaned by resident cleaners.

20. Feeding behavior varies significantly among individuals of at least some species. Thus the habits of an individual can be more limited than those descriptive of its species or even its own population.

ACKNOWLEDGMENTS

Lloyd D. Richards assisted me in collecting the data for this study. I thank Roger F. Cressey and Thomas Bowman, U.S. National Museum, who identified the parasitic copepods and isopods, respectively, around which much of this study is centered. I thank, too, Carl L. Hubbs and Richard H. Rosenblatt, Scripps Institution of Oceanography, and John R. Hunter, U.S. National Marine Fisheries Service, for helpful comments on a draft of the manuscript.

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GRAY WHALES, *Eschrichtius robustus*, AVOID THE UNDERWATER SOUNDS OF KILLER WHALES, *Orcinus orca*

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ABSTRACT

Underwater sound playback experiments were undertaken to determine if gray whales would avoid the sounds of killer whales. When presented killer whale "screams" from an underwater projector, the gray whales swam directly away from the sound source. Controls of no intended stimulus, pure tones, and random noise generally failed to induce an avoidance. It appeared that gray whales localized the killer whale sounds and avoided them as a sign of potential danger.

Killer whales, *Orcinus orca*, are known to attack large marine mammals, including gray whales, *Eschrichtius robustus* (Scammon, 1874; Morejohn, 1968). Several accounts of such attacks were related to the first author by observers who witnessed these events. Among these were Dr. Carl L. Hubbs of Scripps Institution of Oceanography, University of California, and Alan Baldrige, Hopkins Marine Station, Stanford University. In January 1952, Dr. Hubbs saw a gray whale that swam into the thick kelp beds off La Jolla, Calif., apparently fleeing from a group of killer whales. Off the Point Lobos State Reserve, Carmel, Calif., Baldrige observed a gray whale calf being eaten by six to seven killer whales. Other observers had seen the initial attack which also involved the mother whale. When Baldrige arrived, the killer whales were chewing on the lips, tongue, and throat of the dead young gray whale.

Killer whales produce a variety of underwater phonations, including high pitched "screams" and trains of well-separated clicks (Schevill and Watkins, 1966). Underwater sounds have been recorded from about one-half of the known species of marine mammals, and probably all are capable of some vocal behavior. However, except for the echolocating abilities of porpoises and a few of their own behaviors associated with sound production, very little is known about the

significance of underwater sound to marine mammals—virtually nothing where the large whales are concerned.

Taking advantage of the gray whales' migration in nearby waters, we conducted underwater sound playback experiments to find out if they would react to the underwater sounds of killer whales, possibly avoiding them as a sign of danger.

METHODS

The experiments took place off Point Loma, San Diego, Calif., during successive migrations of gray whales in January 1969 and 1970. Each year about 11,000 of these whales pass San Diego on their southward migration to the breeding grounds off Baja California and the Mexican mainland (Rice, 1970). The whales, some with calves, return to our waters in the early spring on their way back to the Bering Sea and the Arctic Ocean.

Field work was done from a large catamaran, RV *Sea See*, which served as a stable and roomy platform, moored in 30 m of water, 33 m seaward of an extensive kelp bed. The ship held a northerly heading at the mooring. Gray whales normally funneled through this location staying relatively close to the coast, but avoiding the thick kelp.

Three kinds of acoustic stimuli were prepared on magnetic tape—a natural sequence of "screams" from killer whales, originally record-

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ed off Dabob Bay, Wash.; a matching sequence of two simultaneous pure tones of 500 and 2000 Hz that resembled the major frequency components in most of the recorded killer whale "screams" and with the same on-off times as the "screams"; and a similarly timed sequence of random noise in the band from 500 to 2000 Hz. Random noise and pure tone were used as control stimuli in addition to a third control wherein there was no playback or any intended stimulus, and the whales were allowed to pass relatively undisturbed. The ship's equipment was silenced throughout the experiments and we made no unnecessary noises.

The sound projector (designed and built at our laboratory) was lowered to 12 m below the surface of the water. It was powered with a 250-w amplifier (Optimation)² connected to the tape recorder (Uher 4200) used for playback. We monitored the output from a receiving hydro-

phone (Wilcoxon, Type M-H90-A) located at the same depth as the projector, 12 m away. Calderon and Wenz (1967) have described this calibrated monitoring system. Underwater signals picked up by the receiving hydrophone led to one track of another Uher stereo tape recorder. The other track carried our running commentary of the whales' behavior. Peak source level of the killer whale signals (Figure 1) and the playback control stimuli was nearly constant—151 db re $1 \mu\text{Newton/m}^2$ ($= 51 \text{ db re } 1 \text{ dyne/cm}^2$) at 1 m in the 1969 experiments, and 176 db in 1970. Because of the natural propagation losses measured in the area, we expected sound pressure levels of the projected sounds to reach the prevailing ambient sea noise level in the third-octave band at 500 Hz, at about 1100 to 1400 m.

² The use of trade names is merely to facilitate description; no endorsement is implied.

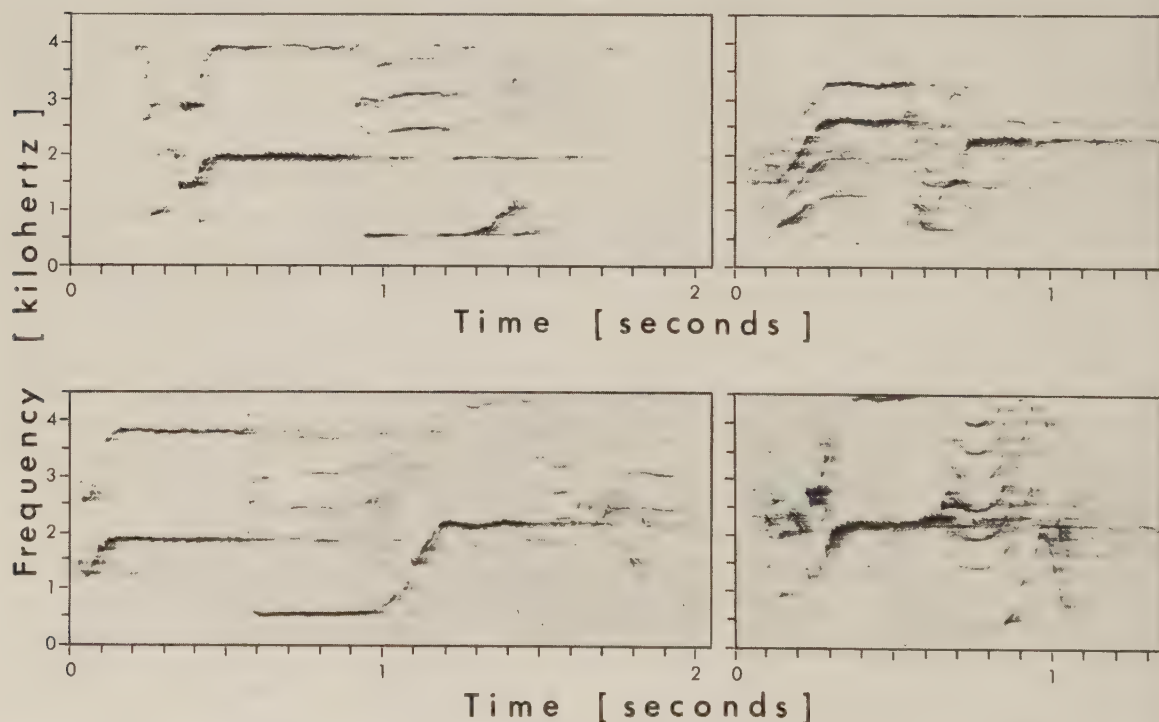


FIGURE 1.—Spectrograms of killer whale "screams" that were played back to migrating gray whales. Of 141 signals on the playback, 83 were very similar to the one at top, left. The others shown are in clockwise order according to their frequency of occurrence on the tape (30, 19, 9). The analyzing filter bandwidth was 20 Hz.

Besides no playback, used in the 1969 experiments, random noise and pure tone served as additional controls in 1970.

Gray whales come by Point Loma at any time during the migration season, day or night, as individuals or in small groups of two to five animals. However, for the total of 5 weeks at sea, we had to work in bright daylight, 0830 to 1630 hr, because of earlier difficulty in seeing the whales in subdued light. Undisturbed whales moved along in a southerly course at average speeds of 10 km/hr, blowing from one to three times every 1 to 14 min. We have also seen killer whales off Point Loma.

Migrating gray whales produce underwater, low-frequency moaning sounds in a band from 20 to 150 Hz (Cummings, Thompson, and Cook, 1968). These sounds last 1.0 to 1.7 sec.

We began an experiment upon seeing an on-coming whale or group of whales that was not encumbered with small boat traffic. Playbacks were generally started when the whales were 150 to 450 m away, toward the north. The experiments were alternated so that successive contacts would not encounter the same situation. Sessions lasted 30 to 100 min, after which the whales had passed the ship or disappeared. There were 77 experiments (77 contacts) involving a total of 132 gray whales. The appearance of "whale-watching" or fishing boats invalidated our work with numerous other contacts. They not only interfered with the whales' progress, but their underwater sounds considerably reduced the playback signal-to-noise ratio.

EXPERIMENTAL RESULTS

The reactions of gray whales to the projected killer whale sounds were spectacular. Blowing whales, or those running at the surface, immediately swirled around and headed directly away from the killer whale sound source. We have no idea of how quickly the submerged whales reacted, but subsequent surfacings were away from the killer whale sound source.

Most whales fled towards the north. However, if their previous southerly course had taken them seaward of the ship and the sound source,

they turned and swam toward the open sea. Gray whales coming from the general direction of the kelp, or those very close to it, fled into this heavy growth and stayed in one general location until we stopped the killer whale stimulus.

Whales came about and resumed a southerly course for Mexico 5 to 30 min after the killer whale sound stopped. If we renewed the playback, before the whales passed by, they again fled northward from the sound source. On several occasions, we repeated the experiments on the same contact up to three times, each with similar results.

Of the 36 contacts principally involving killer whale playback, 30 avoided the sound source, 3 were questionable because we never saw them after playback, and 3 passed the ship as normal, without an apparent avoidance (Table 1). However, one of the last-mentioned was a single whale that had already come very close to the east-west line arbitrarily set on the projecting transducer. This whale could have just as easily avoided the sound source by swimming to the south.

TABLE 1.—Reaction of gray whales contacted on 77 occasions to various stimuli.
[1-4 whales in a contact; 132 whales in all.]

Reaction	Random noise	Pure tones	No playback	Killer whale "screams"
Avoidance	2	2	0	30
No avoidance	8	8	21	3
Questionable	0	0	0	3

All 21 contacts not encountering an intended stimulus (no playback) moved along towards the south in normal fashion. Two contacts of the 10 that received pure tone avoided the sound source. However, 1 of these 2 turned only momentarily. It then resumed the southerly course, still in the presence of the playback stimulus. Of the 10 contacts presented with random noise, 2 avoided by turning towards the north for a very short distance. However, both of these gray whales seemed to be startled only for the moment; then quickly turned a second time and swam down past the random noise sound source.

Two of the 8 contacts that did not avoid random noise and 2 of the 8 that did not avoid

pure tone (Table 1) did flee the killer whale source when we projected it to them before they passed by the sound projector. We did not project killer whale sound to the other contacts that did not avoid random noise and pure tone.

Observers on board noted how little of the avoiding whales' bodies showed above the surface and their unusually small surface disturbance. In many instances their blows were invisible and even blows at close range were scarcely audible. In contrast, the surfacing of undisturbed whales involved the simultaneous appearance of head and blow accompanied by a well-defined surface wake. Their blows were generally visible, and they were audible at close range. After their first appearance, the undisturbed whales showed their backs and sometimes tossed their flukes high into the air. On the other hand, it was difficult to spot the fleeing whales.

We thought that if gray whales associated the projected killer whale "screams" with potential danger, they would have left the area silently to lessen their chance of detection. For example, Schevill (1964) noted that belugas, *Delphinapterus leucas*, became silent in the vicinity of two killer whales. Upon analyzing our data, it turned out that only 2 gray whale phonations appeared on the tapes during killer whale periods, whereas 47 occurred during the control periods.

Gray whales on the breeding grounds frequently exhibit a behavior termed "spying-out" (Gilmore, 1961) wherein the head comes vertically out of the water for several seconds. "Spying-out" in the breeding lagoons seems to be associated with searching for channels; it is also done when the whales are pressed by small boats (communication from Dr. Joseph R. Jehl, San Diego Natural History Museum). Since we have seen this behavior over the past 6 years only three times, we assume that migrating gray whales rarely "spy-out." However, gray whales in the present experiments inevitably "spied-out" after fleeing into the thick kelp following killer whale playbacks.

As a result of these experiments we conclude that gray whales apparently recognize the voice of a killer whale, that they can easily localize

the sounds underwater, and that they flee killer whale phonations probably as a sign of potential danger. Such avoidances consist of several behaviors that appear to function as protective mechanisms—sound localization, silence, rapid escape, reduced exposure, and visual search.

DISCUSSION

Walker (1971) has expressed the opinion that the function of "spy-hopping" ("spying out") by gray whales is not that "... the whale looks around, spying out possible dangers such as ships, or even spotting shore landmarks as aids to navigation." In reply he points out that "In the vertical posture the law of gravity takes over, conveying food from the whale's mouth to a capacious four-chambered stomach. Although a gray whale can swallow when horizontal, the vertical position allows it to clean entangled debris from the filter and to wash food down to the throat for quick ingestion." Walker further reported "... that in the 'spy-hopping' position their [gray whales'] range of vision is limited and that they navigate mostly by echolocation, ..." On the other hand, in the same report is the following portion of a caption to a photograph showing a breaching gray whale. "The catapulting action, he [Dr. Walker] believes, enables the animals to scan the waters around them and make course corrections when interfering noises prevent them from navigating by echo-location."

We are not aware of any experimental evidence that shows whether a gray whale "spys-hops" to look around or to swallow food. Nor is there any substantial evidence that migrating or breeding gray whales feed very much. To the contrary, there is hard evidence (summarized by Gilmore, 1968) that gray whales fast on their way to the breeding grounds, at the grounds, and on the return trip north.

Relative to the killer whale attack cited earlier, Baldrige reported that the mother frequently "spy-hopped" from a distance as the killer whale chewed on her dead calf.

In view of the above, the context of "spy-hopping" exhibited by gray whales during our experiments would seem to imply that this be-

havior had a visual function, i.e., they were probably looking for killer whales. This implication does not necessarily exclude swallowing, but we doubt that the gray whales were feeding under these circumstances.

Moreover, concerning Walker's assertion that gray whales "navigate mostly by echolocation," although it has been clearly demonstrated that porpoises can echolocate underwater objects (reviewed by Norris, 1969), it is not known whether they normally navigate at sea by this means. "Echolocation-like" (Asa-Dorian and Perkins, 1967) and "echolocation" sounds (Poulter, 1968) were recorded in the presence of gray whales, but there was little evidence that the sounds actually were from the whales. There are innumerable sounds in the ocean and many possible sources, but the matching correlations are often difficult to achieve. In any event, we prefer not to use the term "echolocation" in describing the underwater sounds of animals unless they are known to have such a function. Although we have recorded a few clicklike sounds during five seasons of work with gray whales, we have never been able to associate them with the whales. The clicklike sounds that were recorded, while using an array of hydrophones and a technique of computing sound source locations, originated from an area where, apparently, there were no gray whales.

Bioacousticians are not in a position to say with any certainty that gray whales do not echolocate; future experimental work could possibly show that they do. However, our own research with 7 of the 10 species of mysticate whales has yet to reveal any data to suggest that a member of this order uses underwater echolocation.

Whales normally exhale at the surface of the water with the blowholes exposed, but Hubbs (1965) has reported underwater blows by four species of whales—humpback, *Megaptera novaeangliae*; fin, *Balaenoptera physalus*; striped dolphin, *Lagenorhynchus obliquidens*; and the gray whale. In addition, he noted gray whales to refrain from spouting, skip an inhalation, or barely protrude their blowholes in the presence of killer whales. In the same report, Hubbs associated these behaviors with "strange or

frightening stimuli." We were not close enough to see if fleeing gray whales exhaled underwater, but our other cited observations of gray whales covertly avoiding the killer whale sounds certainly parallel Hubbs' observations.

Unfortunately, there was no record of the killer whales' behavior during the original recordings of the phonations used for playback. We advise others who may be involved with sound playback to use appropriately meaningful signals, whenever possible. For example, underwater sounds that are part of an animal's reproductive behavior may have little or no effect on nonbreeding animals. Likewise, feeding sounds may not affect animals that are in a state of alarm. If possible, we would have compared the results of playing back two sets of killer whale sounds in the present experiments—one recorded from attacking whales in a predator-prey situation, if indeed killer whales utter sounds at this time, and another from killer whales that apparently were not feeding or preying.

Based on the methods of this study, our co-workers, Dr. James F. Fish, and John S. Vania subsequently used killer whale sound playback to keep white whales, *Delphinapterus leucas*, from entering the Kvichak River, Alaska, where they eat young salmon before the fish can get to the open sea (Fish and Vania, 1971).

ACKNOWLEDGMENTS

We thank Dr. James F. Fish for helping with the field work and reviewing the report; Bruce C. Parks and Robert Z. Hester for operating the research vessel; Richard N. Bray, George E. Lingle, and John B. Webster for assisting with the data analysis; and Shelley L. Steahl for helping to prepare the manuscript.

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KILLER WHALE, *Orcinus orca*, SOUNDS REPEL WHITE WHALES, *Delphinapterus leucas*

JAMES F. FISH¹ AND JOHN S. VANIA²

ABSTRACT

This study was conducted to determine if the migration of white whales up the Kvichak River, Bristol Bay, Alaska, could be stopped by playing high-intensity underwater sounds to them. While in the river the whales feed on salmon smolt migrating down to the sea. Transmission of killer whale sounds was found to be an effective means for keeping the whales out of the river. During control periods when sound was not projected, the whales moved freely in and out of the river. A permanent play-back system could be installed with little difficulty and would result in a significant reduction in the number of smolts consumed by belugas in the Kvichak River.

White whales, or belugas, *Delphinapterus leucas*, commonly travel 20 to 30 km up Alaska's Kvichak River (Figure 1) on the flood tide and back down on the ebb, foraging on available food

organisms along the way. This twice-daily movement of from 50 to over 500 whales occurs during May and throughout most of June. It ceases only after boat traffic from the seasonal salmon fishery becomes heavy.

The Kvichak River supports the most extensive run of red salmon, *Oncorhynchus nerka*, in the world. These runs range from 1 to 45 million fish per year. Lake Iliamna, the largest lake in Alaska, located about 80 km up the Kvichak, is the principal rearing area for the young salmon before they migrate to the sea as 1- or 2-year olds. The annual migration of smolt occurs during the end of May and the first 2 weeks in June with the peak of migration occurring about the first of June.

Field studies by the Alaska Department of Fisheries (1956) in the early 1950's showed that beluga predation on the salmon smolt occurred when the young fish were migrating to sea and appeared to be most extensive in the confines of the river. As the smolt moved into Kvichak Bay, they scattered and became less vulnerable to predation. Attempts were made to keep the belugas out of the river by chasing them with motorboats and by dropping small charges of explosives into the river. These methods were not very successful and were difficult to use during inclement weather or at night.

From 1963 to 1968 Vania projected various sounds, including killer whale sounds, noise, and music, underwater to the belugas in an attempt



FIGURE 1.—Location of the study area.

¹ Naval Undersea Research and Development Center, San Diego, Calif. 92132.

² Alaska Department of Fish and Game, Anchorage, Alaska 99502.

to keep them out of the river. There was no reaction to the noise or music. The killer whale sounds were only partially successful, apparently because the playback level was too low. His equipment was not designed for underwater sound transmission. Most other workers attempting to influence the movement of wild whales with sound have been unsuccessful. However, in nearly all of these experiments the projected sounds did not exceed 140 to 150 db, re $1 \mu\text{Newton/m}^2$ ($= 40$ to 50 db, re 1 dyne/cm^2) at 1 m. One exception was a sound playback experiment on California gray whales, *Eschrichtius robustus*, by Cummings and Thompson (this issue of *Fishery Bulletin*) where a high-power transmitting system was used and the whales did react to the sounds. Their transmitting system was similar to the high-power system described here which we used to transmit killer whale sounds to belugas in the Kvichak River in June 1970.

METHODS

The primary high-power transmitting system, operated from Station A (Figure 2), consisted of a Uher tape recorder,³ a small impedance-matching preamplifier, a 250-w Optimization power amplifier, and a sound projector developed and built by the Naval Undersea Research and Development Center. Frequency response of this system was ± 3 db from 250 to 4000 Hz, limited by the projector. A secondary, battery-operated playback system, operated from a small boat at Station B (Figure 2), utilized a Uher tape recorder, a 40-w Bogen amplifier powered by a motorcycle battery, and a J-9 sound projector; system response was ± 3 db from 200 to 6000 Hz.

A calibrated recording system, consisting of a Wilcoxon hydrophone, a Uher tape recorder, a sonar calibration box, and a modified GR Octave-Band Analyzer, was used to measure the sound pressure level of the playback signals at various points throughout the river and to record the vocalizations from the belugas. This system was described by Calderon and Wenz (1967).

³ The use of trade names is merely to facilitate descriptions; no endorsement is implied.

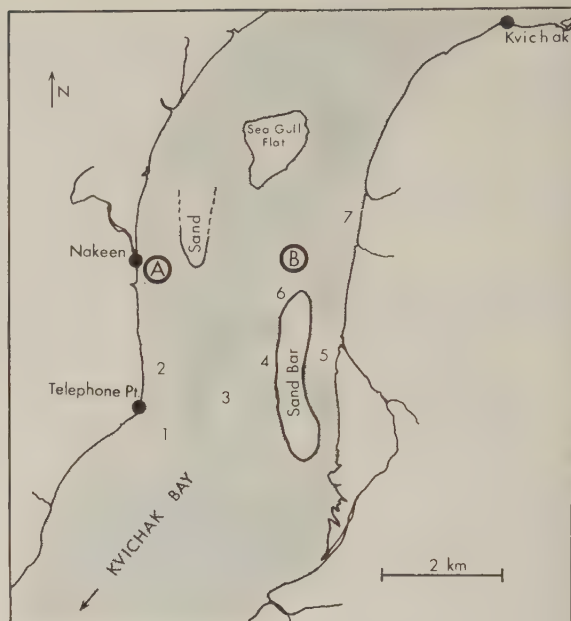


FIGURE 2.—Enlarged drawing of the study area. Transmitting stations were located at A and B. The regions indicated by numerals are locations where playback sound pressure levels were measured.

We selected killer whale, *Orcinus orca*, vocalizations for transmission because of their outstanding acoustic properties and because killer whales are known to kill and eat the relatively slow-swimming white whales (Scammon, 1874: 92; Dergerbøl and Nielsen, 1930; Kleinenberg et al., 1964: 292). A long-play tape was made from "screams" and clicks recorded from killer whales at sea. We do not know, however, what behavior was associated with their sound production.

In the first 10 playback trials, the sound, transmitted only from Station A, was not turned on until the approaching belugas were sighted. But in subsequent trials, when we transmitted from both A and B, we started the playback as soon as the tide changed. Weather and light permitting, a new trial started with each flood tide and lasted from 15 min to 2 hr—as long as necessary to either move the whales back down the river (in those trials where they were permitted to move part way up before the sound

was turned on) or keep them from moving up the river (when the sound was turned on before the whales started upriver).

We had an excellent view of the river from the third floor of a building about 25 m above the water at Station A. Frequent low-altitude flights in small planes provided a check on the exact location of the whales when they could not be seen from the building. The white-colored whales were easy to see in the muddy brown river.

In all, there were 14 playback trials plus 7 control trials in which the whales were observed but sounds were not transmitted. The number of whales involved in each trial ranged from 50 to near 500 with about 100 being the average group size. Because several years of observations indicated the belugas move up the river on every incoming tide, we did not feel it was necessary to have an equal number of transmission and control trials. Consequently, we introduced a control after no more than four successive transmissions, primarily to determine if the belugas had stopped using the river as a result of the sound playbacks.

The sound pressure level of the playback sounds transmitted from Station A was measured at 6 locations in the river within 4 km of the source (Table 1). All levels reported in this paper are in db, re $1 \mu\text{N}/\text{m}^2$. Sandbars prevented good transmission across the river, particularly during the early stages of the flood tide when some of the bars were covered by less than 0.5 m of water. For example, the sounds projected from Station A were below the background noise level at Location 6 (Figure 2). The

best transmitting channel was downriver toward Telephone Point (Location 2), where a level of 132 db was measured for the playback signals. Beyond this area, the signal level dropped off quickly because of the shallow water which extended out a considerable distance from the Point. Most of the energy of the playback sounds occurred in a frequency band from 500 to 5000 Hz; thus we measured ambient levels in this band in addition to making broadband ambient measurements.

RESULTS OF PLAYBACK EXPERIMENTS

In the first seven transmission trials, we let the whales move up the river on the west side to within approximately 2 km of the sound source before turning on the playback. The first six times, the animals turned immediately when the sound began and swam directly out of the river against the strong incoming tide. Their rate of blowing increased and they spent more time at the surface of the water, making it very easy for us to observe their course. On the 7th trial, some of the animals crossed to the opposite side of the river when the sound started and swam downstream along the sandbar (Location 4). The others remained just off Telephone Point for an hour, then moved out of the river.

It was difficult to see the approaching belugas on the 8th trial because of low light, and they had already moved up to Location 6 before we started transmitting. About half of the 100 whales continued up the river; the others turned and swam back out along the sandbar. On the next trial, the animals were first seen moving up the river along the sandbar. Possibly they learned from the previous eight transmissions to avoid the side of the river where the sound projector was located. They continued up to the end of the bar, even after the sound was turned on, but then rounded it and moved back down the river on the other side of the bar (Location 5).

The belugas similarly rounded the upriver end of the sandbar on the 10th trial; but, instead of turning around, they swam to the shore (Location 7) and continued up the river very close to the bank. Poor transmission to this location

TABLE 1.—Sound pressure level (db re $1 \mu\text{N}/\text{m}^2$) of playback signals and ambient noise at the indicated locations.

Location, Fig. 2	Signal broad- band	Ambient noise	
		Broad- band	500-5000 Hz band
	db	db	db
1 m from Source A	170	120	No data
1	115	103	96
2	132	90	84
3	107	91	85
4	103	95	90
6	Below ambient	98	95

from Station A and the reaction of the whales during the 10th trial made it necessary to use a second transducer simultaneously at Station B. A copy of the Station A playback tape was used at Station B, but it was not possible to synchronize the playback sounds from the two projectors. In the subsequent four trials, we turned on the playback as soon as the tide changed, thus eliminating the possibility of the whales going by us unnoticed before the start of transmission. The whales stayed at least 1.5 km down the river from the sound sources during the four transmissions using both projectors.

In each of the seven control trials, when no sound was played, the whales moved directly up the river with the incoming tide, past the transmitting stations. They behaved the same as they did before any of our experiments, with many individuals passing near the pier at Station A. However, we could see very few whales from the viewing station during the killer whale playbacks. Low-altitude reconnaissance flights confirmed our suspicion that numerous whales were remaining well down the river out of view during the transmissions.

Belugas vocalize extensively (Schevill and Lawrence, 1949; Fish and Mowbray, 1962). We recorded their sounds many times at various places throughout the river, often under ideal conditions of flat-calm water with no boat traffic. Once, we drifted in a small boat for over 2 hr during a no-playback control with a group of about 500 whales and recorded a spectacular variety of vocalizations from this relatively undisturbed herd. The belugas emitted very few sounds, however, when the killer whale signals were being transmitted. Quieting of belugas in the presence of killer whales was noted by Schevill (1964).

CONCLUSIONS AND RECOMMENDATIONS

Our experiments showed that playback sounds of killer whales can be used to keep belugas out of Alaska's Kvichak River. This method was very effective and practical. Installing and maintaining such a playback system for 2 to 3

weeks each year would seem to be an economically feasible way of reducing beluga predation on red salmon smolts. Such a system could be started automatically at the beginning of the flood tides and, if necessary, left on for their duration, both day and night. It would not be seriously affected by adverse weather conditions, except for a possible reduction in the range over which the belugas could hear the sounds—because of higher ambient noise levels in the river from wind and rough water.

We recommend using two sound projectors, properly situated to provide good signal levels all the way across the river. Initially, our trials with one projector stopped the whales, but the belugas eventually learned to go up the opposite side of the river where the playback signals were very weak or nonexistent. There were too many sand bars in the river to achieve good signal levels across the river with one projector, regardless of its source level.

Although we only used the two-projector system for four trials, we do not feel the whales would habituate and ignore the playback sounds with continued use. The avoidance of the belugas to this system was striking. The fact that the whales began swimming up the river again after nine trials using the single projector was due, we feel, to their learning to avoid the source, rather than to habituating to the sounds. With sound projectors located on both sides of the river there is probably no channel where the whales can go up the river without hearing the playback sounds. On the other hand, even if the belugas were to habituate after 2 weeks of playback, this would not significantly affect the usefulness of the technique. Playback could be timed to coincide with the 2-week peak of the smolt run.

After completing this experiment with killer whale playbacks, we projected a 2500-Hz continuous tone and 2500-Hz randomly pulsed tones. The belugas continued up the river during the continuous-tone playback, but turned back on the two occasions when pulsed tones were transmitted. Since these playbacks were tried after the white whales had been subjected to the killer whale sounds for 2 weeks, we cannot speculate on how naive whales would have reacted.

More experiments are necessary before we can conclude whether or not the belugas recognized the killer whale sounds as such.

ACKNOWLEDGMENTS

We thank Dr. William C. Cummings for his suggestions on the manuscript, George E. Lingle and John B. Webster for assisting with the data, and the New England Fish Company for providing laboratory space and aiding with the field work. Fish's work was supported by Independent Research funding of the Naval Undersea Research and Development Center during his appointment as a NRC-NUC Postdoctoral Research Associate, and Vania's in part by Federal Aid to Wildlife Restoration Funds, Project W-17-R.

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DEVELOPMENTAL ABNORMALITIES OF THE FLATFISH *Achirus lineatus* REARED IN THE LABORATORY¹

EDWARD D. HOUDE²

ABSTRACT

Of 31 *Achirus lineatus* juveniles reared in a single experiment, 26 were abnormal. Abnormalities included incomplete eye migration, hooked dorsal fins, the presence of a left pectoral fin, ambicoloration, and partial albinism. The abnormal specimens have been described and photographed. A single reversed specimen, preserved as a nearly metamorphosed individual, also is described. Most of the abnormal conditions were interrelated. Possible effects of the rearing tank environment on abnormal development are discussed.

Larvae of lined soles, *Achirus lineatus* (Linnaeus), were reared in the laboratory from fertilized eggs collected in plankton tows from Biscayne Bay, Fla. Development and details of metamorphosis were described by Houde, Futch, and Detwyler (1970). No developmental abnormalities were detected until metamorphosis was nearly complete. At 50 days after hatching, the 31 survivors were examined and 26 (84%) were found to be abnormal.

The unique metamorphosis of flatfishes (Pleuronectiformes) may be responsible for the high percentage of abnormal specimens reported for this group in the literature (Norman, 1934; Hubbs and Hubbs, 1945; Dawson, 1962). Most of the described abnormal conditions in flatfishes were encountered in lined soles from this rearing experiment. Abnormalities included, in order of frequency, (1) ambicoloration (25 specimens), (2) retention of left pectoral fin, normally lost after metamorphosis (23 specimens), (3) partial or no migration of the left eye (17 specimens), (4) hooked dorsal fin, (same 17 specimens), (5) partial albinism (1 specimen), and (6) reversal (1 specimen). Similar abnormalities have been described previously in

flatfishes, but are rare in the family Soleidae (Dawson, 1962). None have been reported previously for *A. lineatus*. The extremely high percentage of abnormal individuals among laboratory-reared specimens from this experiment apparently reflects some effect of the aquarium environment on the development of *A. lineatus*. A high proportion of pigment-deficient individuals of plaice (*Pleuronectes platessa*) was associated with high densities of metamorphosed specimens in rearing tanks and with low food levels in the tanks (Shelbourne, 1964, 1965; Riley, 1966), but other abnormalities were not discussed. Although the exact nature of the influence of the rearing tank on the production of abnormal specimens of lined soles is unknown, it is possible that harmful effects are created by (1) frequent contact with sides of small rearing tanks, (2) unnatural lighting, and (3) high concentrations of metabolites. Seshappa and Bhimachar (1955) reported failure of eye migration in the tongue sole *Cynoglossus semifasciatus* (Cynoglossidae) when postlarvae were kept in the dark. Two of my previous rearing experiments with *A. lineatus* also produced abnormal individuals. Some juvenile specimens of other flatfishes, *Paralichthys albigutta* (Bothidae) and *Gymnachirus melas* (Soleidae), also developed abnormally when reared from eggs hatched in my laboratory. The high incidence of abnormalities in the reared lined soles suggests that these conditions in flatfishes are not

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necessarily genetically controlled. When present in significant numbers, abnormalities may have important implications for those considering flatfishes in aquaculture.

METHODS

Rearing techniques were briefly described and larval development through metamorphosis reported by Houde et al. (1970). Methods used to rear *A. lineatus* were similar to those described in detail by Houde and Palko (1970). The 31 juveniles of lined sole were maintained in two 75-liter aquariums and fed on frozen brine shrimp (*Artemia salina*). Before metamorphosis larvae were fed wild zooplankton which consisted mostly of copepods. Beginning 50 days after hatching and at approximately 1-month intervals, growth was determined by measuring fish to the nearest millimeter total length (TL) and then returning them to the aquariums. Individuals were recorded as normal or abnormal when they were measured, the distinction being based on whether the dorsal fin was hooked.

Beginning 137 days after hatching, some fish were sacrificed and preserved for detailed examination. Radiographs were made to study skeletal structures. Specimens were accessioned into the fish collection at the Tropical Atlantic Biological Laboratory.

NORMAL SPECIMENS

Only five specimens were normal in all respects (Figure 1). Meristics and morphometrics of normal individuals fell within the range of variation for the species (Jordan and Evermann, 1898).

GROWTH AND MORTALITY

Growth was compared between grossly deformed specimens with hooked dorsal fins and normal specimens or those whose only anomalies

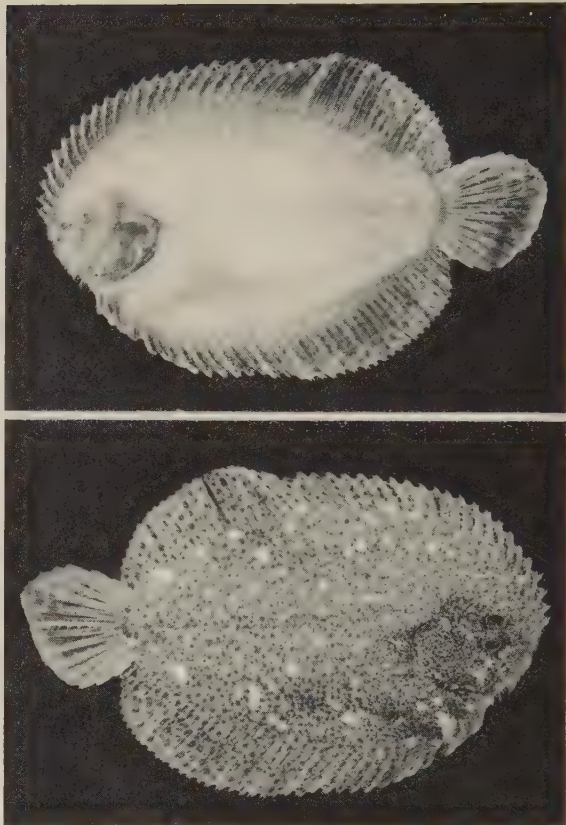


FIGURE 1.—Left side (upper photo) and right side (lower photo) of 45.8 mm TL normal *Achirus lineatus* reared in the laboratory. Dorsal fin was bent when specimen was preserved but it is normal.

consisted of unusual pigmentation and the presence of a left pectoral fin. Specimens with abnormal pigmentation or with left pectoral fins were classified as "normal" at the time they were measured because such conditions could not always be detected in small living juveniles. Although "normal" individuals initially were longer than abnormal ones, some compensation apparently occurred, and little difference in lengths was apparent between specimens still living from the two categories at 250 days after hatching. Both "normal" and abnormal specimens averaged about 52 mm TL at this time. No natural mortality occurred for either normal or abnormal lined soles between 50 and 275 days after hatching.

TABLE 1.—Abnormalities of *Achirus lineatus* reared in the laboratory. A plus symbol indicates the presence of the abnormality and a minus symbol indicates its absence.

Specimen number	Total length	Pigment on blind side		Left pectoral fin	Hooked dorsal fin	Left eye migration
		Body	Head			
	mm	%	%			
64	32.6	100	100	+	+	incomplete
65	27.5	100	100	+	+	incomplete
68	29.5	100	100	+	+	incomplete
69	39.6	100	100	+	+	incomplete
72	45.8	100	100	+	+	incomplete
73	41.6	100	100	+	+	incomplete
84	42.7	100	100	+	+	incomplete
85	48.0	100	100	+	+	incomplete
88	60.0	100	100	+	+	incomplete
91	52.0	100	100	+	+	incomplete
92	50.0	100	100	+	+	incomplete
93	53.0	100	100	+	+	incomplete
67	34.8	100	100	+	+	nearly complete
83	43.8	100	100	+	+	nearly complete
87	61.0	100	100	+	+	nearly complete
90	77.0	100	100	+	+	nearly complete
75	58.4	100	100	+	—	complete
79	56.3	100	100	+	—	complete
89	69.0	100	100	+	—	complete
66	45.5	100	~70	+	—	complete
74	54.8	100	~70	+	—	complete
63	39.1	100	~10	+	—	complete
78	52.1	100	~10	+	—	complete
70	48.1	100	~10	—	—	complete
77	54.5	~90	0	—	—	complete
186	66.0	0	0	—	+	no eye migration
71	45.8	0	0	—	—	complete
76	63.6	0	0	—	—	complete
80	42.6	0	0	—	—	complete
81	40.3	0	0	—	—	complete
82	52.9	0	0	—	—	complete

¹ This specimen had a small albinistic area on the right side of its head.

ABNORMALITIES

PIGMENTATION

Among the many abnormally pigmented specimens (Table 1) four major categories could be distinguished. Three were types of ambicoloration and the fourth was partial albinism: (1) Nineteen specimens were completely pigmented on both sides (Figures 2 and 3). (2) Two specimens were completely pigmented except for the mouth region on the left (blind) side (Figure 5). (3) Four specimens were completely or almost completely pigmented on the body of the blind side but not on the head, which had little (10%) or no pigment (Figure 6). (4) One specimen was partially albinistic on the right side of the head, but other pigmentation was normal. The

eye completely failed to migrate in this specimen (Figure 7). Normal coloration (five specimens) consisted of a lack of pigment on the blind side, except for tiny melanophores scattered over the posterior third of the body (Figure 1). All except two ambicolored individuals had some other associated abnormality, but normally pigmented specimens were always normal in other respects.

LEFT PECTORAL FIN

The left pectoral fin disappeared from normal lined soles when metamorphosis was nearly complete (Houde et al., 1970). A fin with 1 to 6 rays remained on 23 of the 31 juveniles from the rearing experiment. All specimens with a left pectoral fin also were abnormally pigmented.

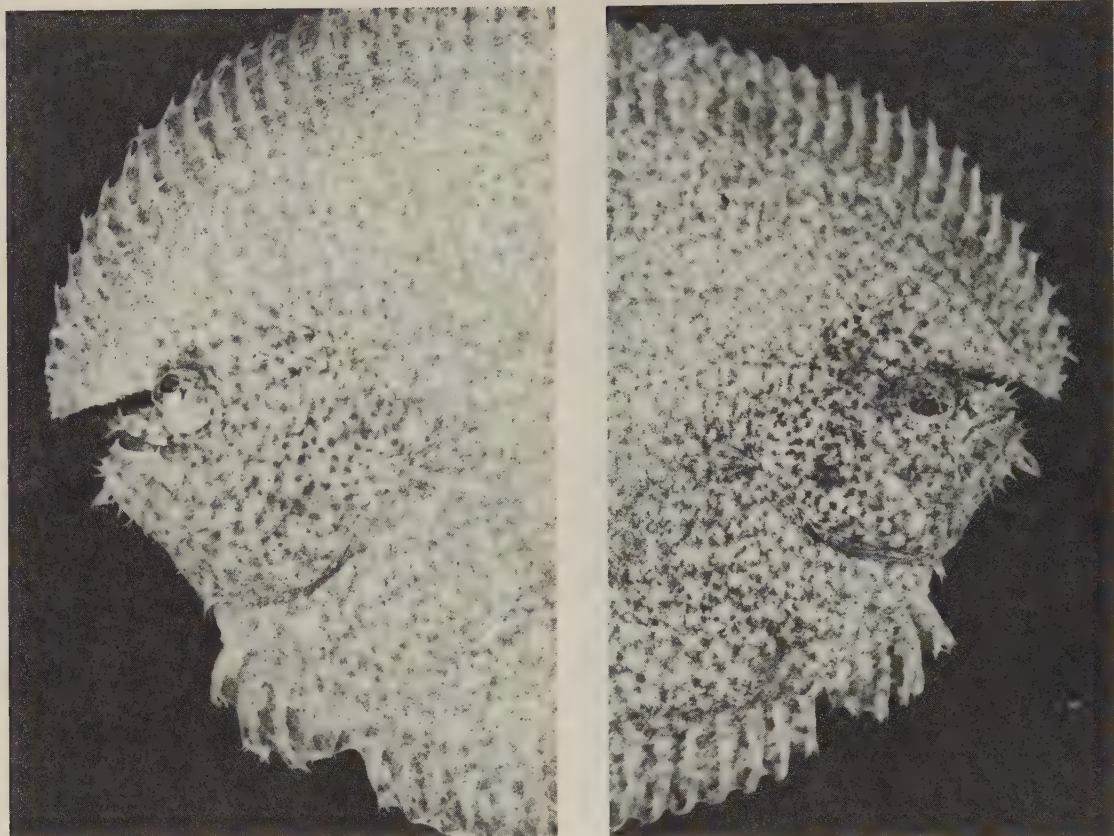


FIGURE 2.—Left side (left photo) and right side (right photo) of head of 39.6 mm TL abnormal *Achirus lineatus* reared in the laboratory. Ambicoloration, hooked dorsal fin, incomplete eye migration, and presence of a left pectoral fin.

Usually the fin was associated with a hooked dorsal fin and failure of eye migration (Figures 2 and 3), but a pectoral fin also was present on some individuals without those abnormalities (Figure 5).

HOOKED DORSAL FIN

Seventeen specimens had a hooked dorsal fin. In normal metamorphosis, the left eye of *A. lineatus* migrates across the dorsal midline under the projecting dorsal fin; the "hook" of the dorsal fin subsequently grows down toward the snout, eventually becoming adnate to the head. Development of the dorsal fin during metamor-

phosis was described for normal *A. lineatus* by Futch, Topp, and Houde.³ In normal lined soles the first five dorsal fin pterygiophores articulate with serrations in the supraoccipital bone. The three anteriormost pterygiophores are directed anteriorly, lying nearly parallel to the axis of the neurocranium. A fleshy connection is established between the dorsal fin and the prefrontal complex of the neurocranium. Radiographs of abnormal lined soles revealed that rotation of the frontal and prefrontal bones was incomplete during metamorphosis. The anteriormost pter-

³ Futch, C. R., R. W. Topp, and E. D. Houde. Developmental osteology of the lined sole, *Achirus lineatus* (Pisces: Soleidae). (Unpublished manuscript.)

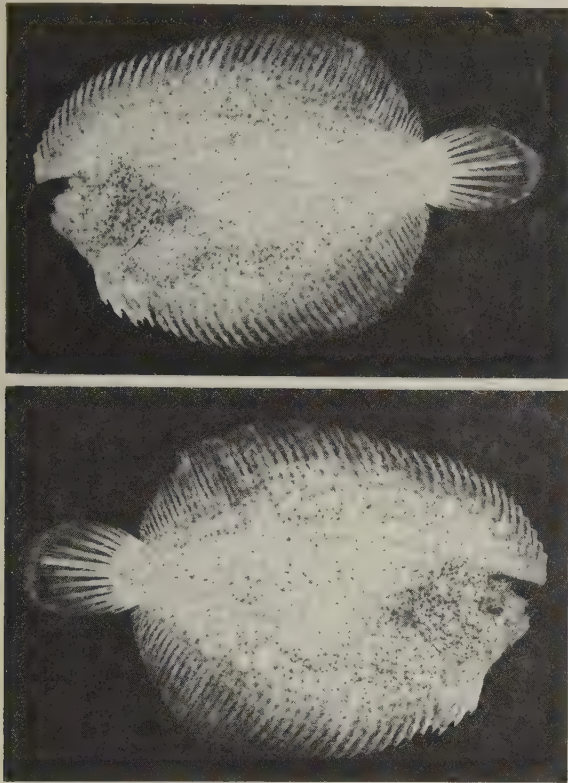


FIGURE 3.—Left side (upper photo) and right side (lower photo) of 32.6 mm TL abnormal *Achirus lineatus* reared in the laboratory. Abnormalities include ambicoloration, hooked dorsal fin, partial eye migration, and presence of a left pectoral fin.



FIGURE 4.—Right side of head of 34.8 mm TL abnormal *Achirus lineatus* reared in the laboratory. Abnormalities include ambicoloration, hooked dorsal fin, and presence of a left pectoral fin. Eye migration nearly complete.

ysiophores appeared normal and articulated with the supraoccipital serrations, but were directed away from the neurocranium at an angle of about 20°. No fleshy connection was established between the dorsal fin and the incompletely rotated prefrontal complex. Hooked dorsal fins and eye migration failure are associated (Figures 2 and 3), but the hooked condition can also be present when eye migration is nearly normal (Figure 4).

EYE MIGRATION

Migration (rotation) of the left eye was incomplete in 17 specimens. The abnormality

ranged from almost total failure of migration (Figures 2 and 7) to various stages of partial or nearly complete migration (Figures 3 and 4). All individuals with this abnormality also had hooked dorsal fins and were ambicolored or partially albinistic. In normal *A. lineatus* eye migration began at about 3.5 mm TL and was complete at 6.5 mm TL (Houde et al., 1970).

REVERSAL

A single reversed specimen was reared. This specimen was not included among the 31 treated in Table 1 because it was preserved before metamorphosis was completed. Reversals among the

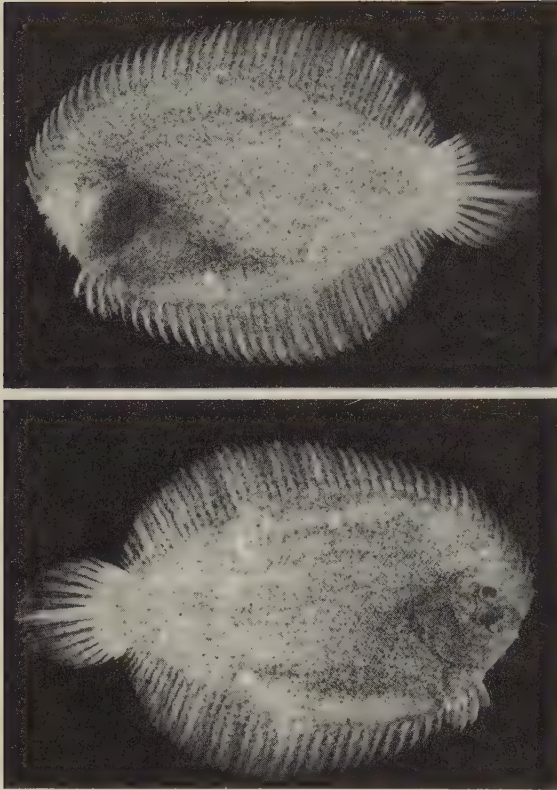


FIGURE 5.—Left side (upper photo) and right side (lower photo) of 45.5 mm TL abnormal *Achirus lineatus* reared in the laboratory. Abnormalities include ambicoloration and the presence of a left pectoral fin.

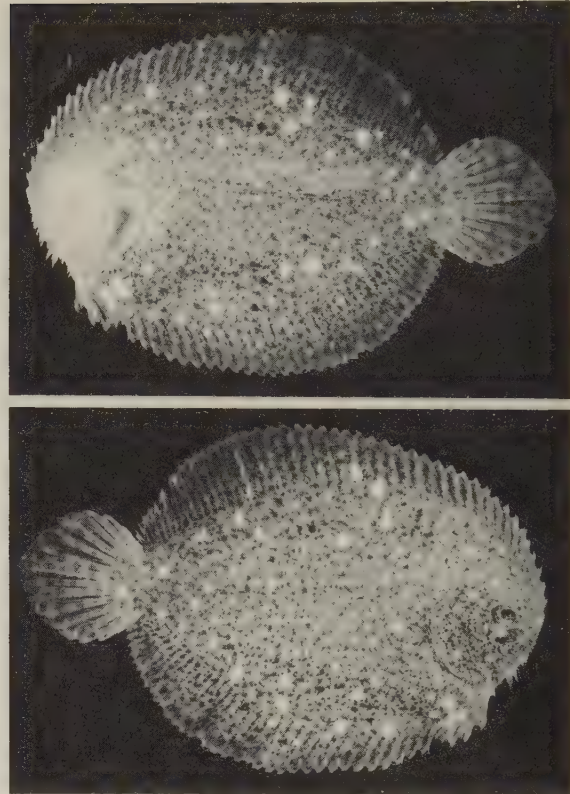


FIGURE 6.—Left side (upper photo) and right side (lower photo) of 48.1 mm TL abnormal *Achirus lineatus* reared in the laboratory. Specimen is ambicolored but otherwise normal.

Soleidae are extremely rare⁴ (Hubbs and Hubbs, 1945). The specimen was a nearly metamorphosed individual of 5.5 mm TL (Figure 8), that appeared normal in other respects when compared with other postlarvae of the same length. Internal organs were not examined to determine whether they were reversed. Normal *A. lineatus* of the same length have been illustrated and described by Houde et al. (1970).

⁴ The photograph in Herald (1961; fig. 139) should not be mistaken as a reversed *Gymnachirus williamsoni*, because in actuality this photograph was produced by an accidental reversal of a Kodachrome slide during preparation of the text.

SUMMARY

Most abnormalities of the laboratory-reared *A. lineatus* appeared related to each other. The presence of related anomalous conditions in individual specimens of flatfishes often has been reported, and the apparent association of ambicoloration, hooked dorsal fins, incomplete eye migration, and the tendency toward symmetry in paired fins has been discussed (Dawson, 1962; Gudger and Firth, 1936; Norman, 1934). The 26 aberrant juvenile specimens in my series of 31 were examined to determine the association of abnormal conditions in individual fish. Examination of Table 1 shows that the abnormalities are associated. Sixteen specimens had

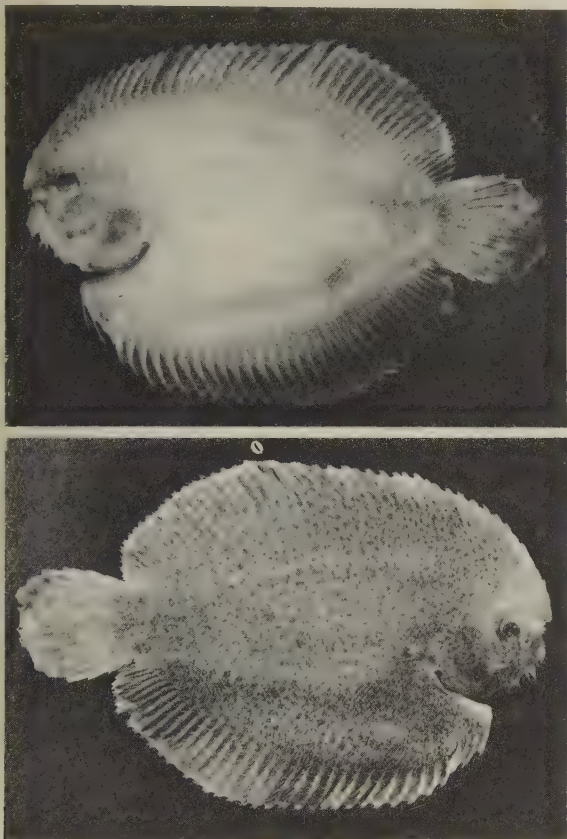


FIGURE 7.—Left side (upper photo) and right side (lower photo) of 66.0 mm TL abnormal *Achirus lineatus* reared in the laboratory. Partial albinism, hooked dorsal fin, no eye migration.

all four major anomalies and 16 to 23 specimens had two or three coexistent aberrancies. The "rule" of Gudger and Firth (1936), that was supported by extensive data on flatfishes (Dawson, 1962), stated that specimens with complete pigmentation of the body and pigmentation covering at least one-quarter to one-third of the head on the blind side will have a hooked dorsal fin and incomplete eye migration. Five of my lined soles fitted that category of ambicoloration but had neither a hooked dorsal fin nor incomplete eye migration (Table 1 and Figure 5). All but two ambicolored specimens also were abnormal in some other respect. Ambicolored individuals in which the body and more than 10% of the

head on the blind side were pigmented retained a pectoral fin on the blind side. Eye migration never was complete in those specimens with a hooked dorsal fin.

A single specimen (Figure 7; #86 in Table 1) was unique in that the left eye completely failed to migrate. A portion of the right side of its head was unpigmented making it the only partially albinistic specimen in the series. The left side, which was unpigmented and lacked a pectoral fin, was similar in these respects to the blind side of normal lined soles. A well-developed hooked dorsal fin was present. Similar abnormalities were present in a naked sole (*Gymnachirus melas* Nichols) that was reared at the laboratory.

The high percentage of abnormalities in laboratory-reared *A. lineatus* must have been influenced by rearing conditions, since abnormal lined soles apparently are extremely rare in nature. Further controlled experiments should make it possible to determine what factors cause abnormal metamorphosis of lined soles and perhaps other flatfishes. These experiments also might test the common assumption that survival of abnormal flatfishes is lower than that of normally metamorphosed individuals, since no advantages in either survival or growth of normal juveniles of *A. lineatus* were detected in the initial rearing experiment.

ACKNOWLEDGMENTS

Assistance in rearing the larvae was provided by Barbara Palko and Robert Detwyler. Charles

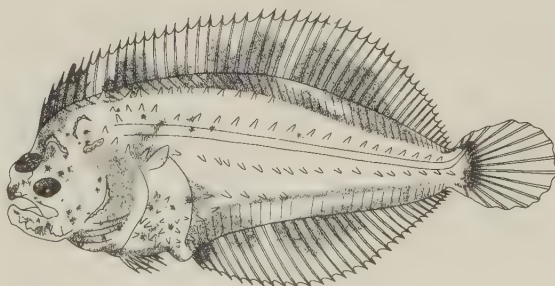


FIGURE 8.—Left side of a reversed, nearly metamorphosed, 5.5 mm TL specimen of *Achirus lineatus* reared in the laboratory.

Futch, William J. Richards, and C. R. Robins have reviewed and criticized the manuscript. The reversed specimen was illustrated by Grady Reinert. Photographs were taken by Andrew Ramsay and Anna Delor.

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THE EARLY LIFE HISTORY OF SKIPJACK TUNA, *Katsuwonus pelamis*, IN THE PACIFIC OCEAN

HOWARD O. YOSHIDA¹

ABSTRACT

This study investigates the early life history of skipjack tuna, including the distribution, abundance, age, and growth. The study is based on 1,742 juvenile skipjack tuna that were found in the stomachs of 6,867 billfishes caught in Hawaiian waters and in the South Pacific by commercial longline boats. The smallest juvenile taken near Hawaii was 5.9 cm in standard length, and in the South Pacific 1.6 cm in standard length. Regressions describing the relations between the standard length and lengths of (1) the vertebral column, (2) the precaudal vertebrae, (3) caudal vertebrae, (4) the 1st-10th vertebrae, and (5) the 21st-30th vertebrae of juvenile skipjack tuna were determined. The regressions provided estimates of the standard length of fragmentary specimens. Juvenile skipjack tuna were widely distributed between lat 5° and 32° S, and long 137° W and the 180th meridian. North of the equator, the commercial longline boats fished close to the main Hawaiian Islands, and thus only a limited picture was obtained of the areal distribution of juvenile skipjack tuna. Juvenile skipjack tuna were found in almost all months in Hawaiian waters. They were most numerous in July and August. In the South Pacific, juveniles were also found in almost all months between lat 5° and 20° S. Peaks in the apparent abundance were evident in April and October in the area north of lat 10° S. Juvenile skipjack tuna appeared to be more numerous in the South Pacific than around Hawaii.

Length-frequency distributions of juvenile skipjack tuna from Hawaii showed well-defined modes, which progressed with time. The growth of the juveniles was estimated by using the modal lengths determined from the monthly length-frequency distributions. Skipjack tuna between 9 and 40 cm around Hawaii are estimated to grow 2.0 cm per month. One-year-old fish are estimated to be 31 cm in standard length.

In 1968, 70,746 metric tons of skipjack tuna, *Katsuwonus pelamis*, were landed in the eastern Pacific (Inter-American Tropical Tuna Commission, 1970) and 109,018 metric tons were landed in Japan (Japan. Fisheries Agency, Research Division, 1970). Because of its commercial importance much knowledge has been accumulated on the biology of the skipjack tuna. Information on early life history, however, is incomplete. Matsumoto (1958) described skipjack tuna larvae and their temporal and spatial distribution in the central Pacific. Ueyanagi (1969) reported on the distribution of larval skipjack tuna in the Pacific Ocean between 1960 and 1967, and Higgins (1967) summarized the distributional records of juvenile skipjack tuna in the Pacific.

The present study is based on immature skipjack tuna between 1.6 and 40 cm taken from the stomachs of billfishes near Hawaii and in the South Pacific. Included are observations on geographical and seasonal distribution, length-frequency distributions, and age and growth rates.

MATERIALS AND METHODS

The stomachs of 6,867 billfishes² were examined in this study. Those examined included 4,118 striped marlin, *Tetrapturus audax*; 1,606 blue marlin, *Makaira nigricans*; 383 shortbill spearfish, *T. angustirostris*; 216 sailfish, *Istiophorus platypterus*; 196 swordfish, *Xiphias gladius*; 171 black marlin, *M. indica*; and 177 billfishes that were not identified to species.

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² The term billfishes as used in this paper includes swordfish.

Sixty-six percent of the stomachs came from billfishes captured near Hawaii between July 1962 and April 1966. These stomachs were used previously in an early life history study of albacore, *Thunnus alalunga* (Yoshida, 1968). Most of these billfishes were caught within 37 km (20 nautical miles) of the main islands. A few were caught as far as 740 km (400 nautical miles) from Oahu. Thirty-four percent of the stomachs came from billfishes caught in the South Pacific between lat 5° and 32° S, and between long 135° W and 179° E (Figure 1). These were caught on longline gear by boats operating out of American Samoa between January 1964 and July 1966. The fishery has been described by Otsu and Sumida (1968).

Arrangements were made with the crews of several boats to have billfish stomachs collected on the fishing voyages. Each cooperating crew was provided with a stainless steel tank, formaldehyde solution, labels, and collecting bags. The crew was paid 50 cents for each stomach.

In the laboratory at Honolulu, all tuna and tunalike specimens were sorted from the stomach contents and identified. Skipjack tuna were identified by skeletal characters (Godsil and Byers, 1944). Standard length was recorded for all intact specimens. Following a technique used earlier (Yoshida, 1968), a method was devised to estimate the standard length of fragmentary specimens. Relations were determined between the standard length and the length of: (1) the complete vertebral column (41 vertebrae), (2) the precaudal vertebrae (vertebrae 1-20), (3) the caudal vertebrae (vertebrae 21-41), (4) 1st-10th vertebrae, and (5) 21st-30th vertebrae, based on 77 intact juvenile skipjack tuna specimens from around Hawaii and the South Pacific. All the relations appeared to be linear and straight lines were fitted to the data by the method of least squares. Combining the samples from Hawaii and the South Pacific should not adversely affect the results. A plot of the data for all five relations did not indicate any differences between the North Pacific and South Pacific samples. A covariance analysis applied to the relation between the standard length and the length of the complete vertebral column for Hawaiian and South Pacific juvenile

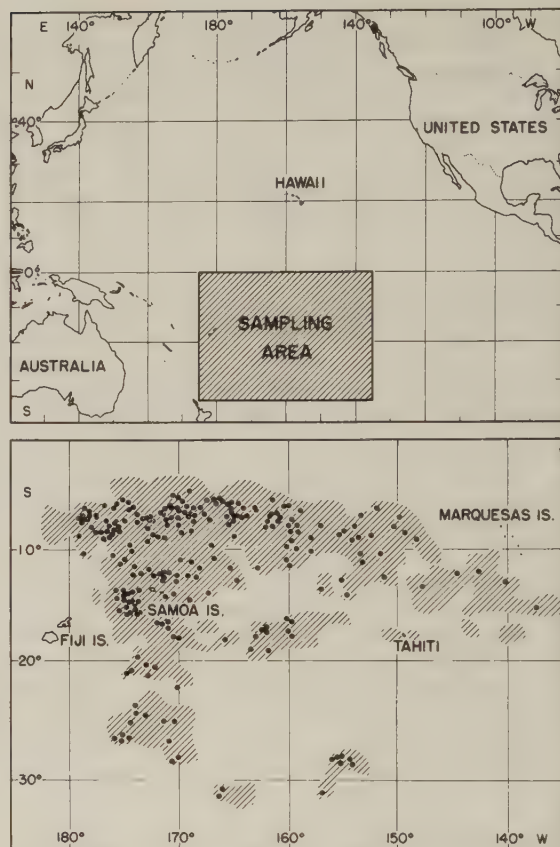


FIGURE 1.—The location of capture of billfishes (shaded area) and the distribution of juvenile skipjack tuna (dots) in the South Pacific.

skipjack tuna confirmed the lack of significant differences between the samples. No significant differences were found in the regression coefficients ($F = 0.105$; $df = 1, 73$) and in the intercepts ($F = 0.053$; $df = 1, 74$). The regressions of the standard length on the various vertebral segments are presented in Table 1.

The lengths of most of the specimens were estimated by using a suitable regression. For 22% of the specimens the relative position of the fragments could not be determined, and the regressions were not used. For these specimens the standard length was estimated by comparing the average length of the vertebrae in the fragment with the average length of the vertebrae

of the specimens used to calculate the regressions.

TABLE 1.—Regressions describing the relations between the standard length and lengths of the vertebral column, precaudal vertebrae, caudal vertebrae, 1st-10th vertebrae, and 21st-30th vertebrae of juvenile skipjack tuna [l = standard length (cm), L = length of vertebral fragments (cm)].

Segment of vertebral column	Regression	Standard deviation from regression
Complete vertebral column	$l = 0.0693 + 1.2262L$	0.435
Precaudal vertebrae	$l = 0.6544 + 2.4926L$	0.439
Caudal vertebrae	$l = -0.4414 + 2.4196L$	0.445
1st-10th vertebrae	$l = 0.4280 + 5.2938L$	0.515
21st-30th vertebrae	$l = -0.2637 + 4.6942L$	0.595

DISTRIBUTION AND ABUNDANCE

Seasonal and areal coverage was spotty, but sampling was extensive enough to permit meaningful analysis for the present study. In the following sections I will discuss the distribution and the seasonal and annual apparent abundance of juvenile skipjack tuna in the South Pacific and near Hawaii.

AREAL DISTRIBUTION

Commercial longline boats engaged in collecting billfish stomachs in the South Pacific ranged over a wide area, and juvenile skipjack tuna, as indicated by their presence in billfish stomachs, also were widespread (Figure 1). Around Hawaii fishing was restricted to a relatively small area, and so a more limited picture was obtained of the areal distribution of juvenile skipjack tuna.

Skipjack tuna larvae are widely distributed in the Pacific Ocean (Matsumoto, 1966; Ueyanagi, 1969). Ueyanagi (1969) reports that larvae were taken across the entire South Pacific between the equator and lat 10° S. Also, west of long 140° W larvae were taken as far south as lat 32° S. My study shows that the distribution of juvenile skipjack tuna is similar to the distribution of the larvae. In the North Pacific, skipjack tuna larvae have been found around Hawaii and across the entire Pacific between the equator and lat 20° N. In the western North Pacific, between long 160° W and the Asian

continent, larvae have been taken almost as far north as lat 35° N (Ueyanagi, 1969).

My study indicates that gaps in the distribution of juvenile skipjack tuna in the Pacific reflect a lack of sampling. Higgins (1967) has a somewhat similar viewpoint. It is likely that the juveniles are as widely distributed as larval skipjack tuna in the North Pacific.

The distribution of juvenile skipjack tuna in the South Pacific by quarters of the year (all years combined) is shown in Figure 2. This is only the apparent distribution, however, because it reflects the operations of Samoa-based vessels. These vessels primarily seek albacore, and therefore they fish the areas where albacore catch rates tend to be high. In the first half of the year, vessels generally operate north of lat 20° S, and in June or July they move as far south as lat 30° S before heading north again (Otsu and Sumida, 1968). Samples were available mostly from north of lat 20° S, and juvenile skipjack tuna were found throughout the sampling range. In the third and fourth quarters, samples were available from a wider area, and again juvenile skipjack tuna were taken from almost the entire area sampled. Although seasonal coverage was incomplete throughout the total area, synoptic sampling would probably produce juveniles in all seasons and throughout the total area.

SEASONAL APPARENT ABUNDANCE

Hawaii

Apparent abundance is expressed here as number of skipjack tuna per 100 billfish stomachs. The apparent abundance of juveniles around Hawaii, all years combined, is shown in Figure 3. The juveniles were more numerous during July, August, and September. A peak in abundance usually occurred in August. These observations confirm Matsumoto's (1966) conclusion that skipjack tuna in the Hawaiian Islands spawned during the summer. He showed that the abundance of larval skipjack tuna peaked in July.

The apparent abundance of juveniles in 1963, 1964, and 1965 offers interesting contrasts. For example, in August 1963 the apparent abundance peaked sharply to 100 juveniles per 100 bill-

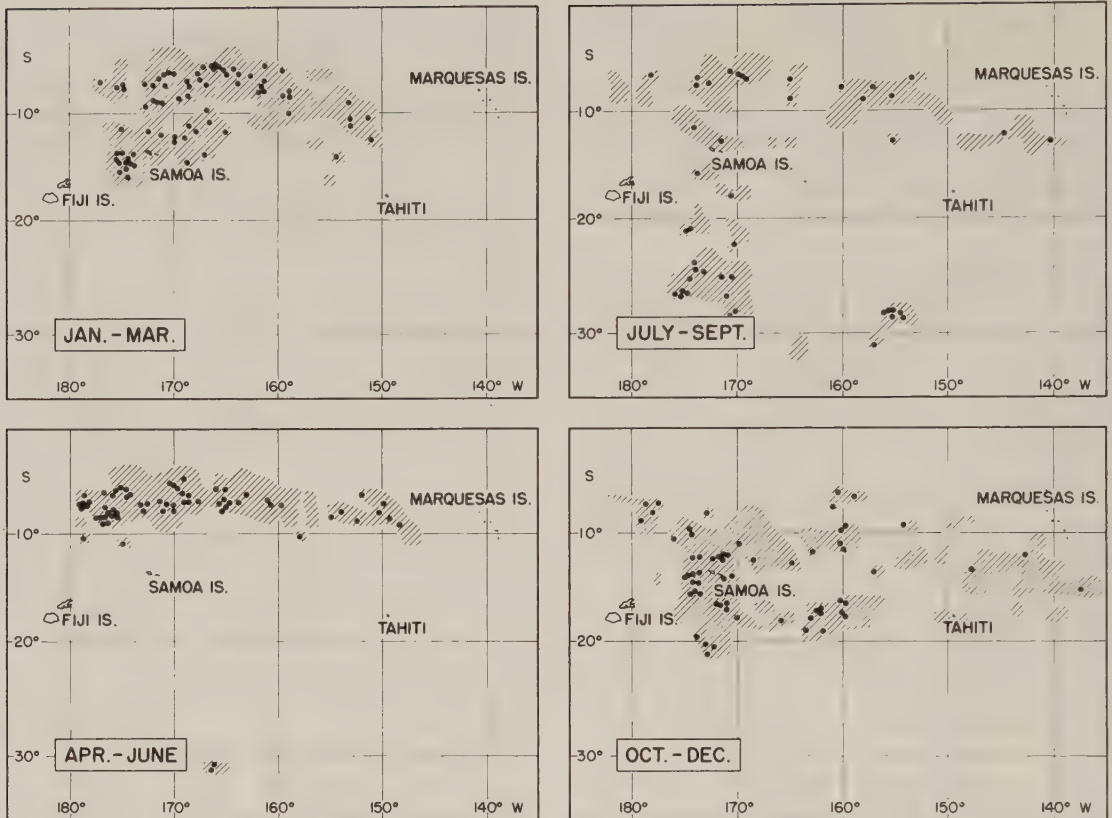


FIGURE 2.—Distribution of juvenile skipjack tuna (dots) by quarters of the year in the South Pacific. The shadings show the billfish sampling area.

fishes. Only small numbers were taken between January and June and between October and December. In 1964 and 1965 the summer peak in abundance was not so high; however, juveniles were more numerous in other months. The apparent abundance of juveniles was highest in 1964 when an average of 21.3 juveniles per 100 billfishes was taken. In 1965, an average of 19.1 juveniles was taken, and in 1963 the average was 12.4.

South Pacific

To examine the seasonal apparent abundance of juvenile skipjack tuna in the South Pacific, the area north of lat 10° S was considered separately from the area between lat 10° and 20° S (Figure 4). Because the coverage was poor in

any one year, the data for all years were combined.

Juvenile skipjack tuna were taken throughout the year north of lat 10° S. Peaks in the apparent abundance were evident in April and October. Larvae have been taken throughout the year in the equatorial waters (lat 10° N to 10° S) of the central Pacific (Matsumoto, 1966). They were most numerous between April and July. Thus, the apparent abundance of the juveniles differs somewhat from that of the larvae in that a peak was absent in larval abundance in the latter half of the year. The difference in apparent abundance between the larvae and juveniles may not be real. The small number of stomachs collected in the latter half of the year may not have been adequate to reveal the true abundance of juveniles.

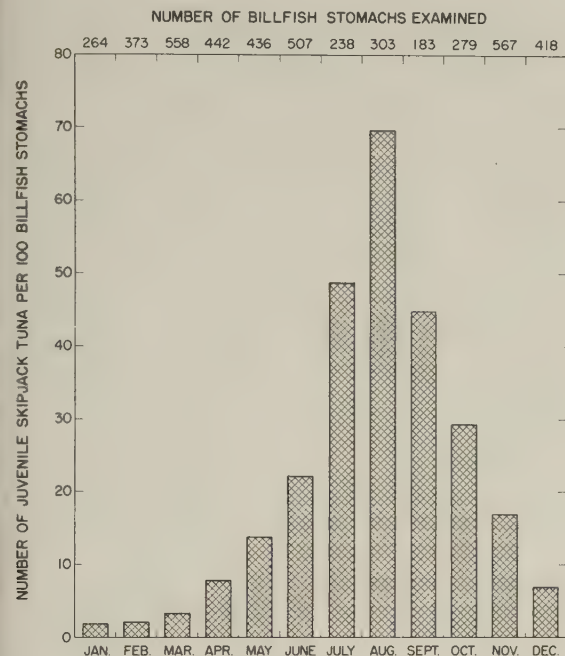


FIGURE 3.—Apparent abundance of juvenile skipjack tuna in Hawaiian waters, 1962-66.

Juveniles were taken in all months except July between lat 10° and 20° S. Here also the uneven sample sizes make the analysis of apparent abundance difficult. It appears, however, that juveniles were numerous from November to February. No comparable data on larval skipjack tuna in this area are available.

The apparent abundance of juvenile skipjack tuna was higher in 1964 than in 1965 in the South Pacific (Table 2). In 1964, juveniles were

TABLE 2.—Annual apparent abundance of juvenile skipjack tuna in the central Pacific Ocean.

Year	Area	Number of billfishes	Number of juvenile skipjack tuna	Number of juveniles per 100 billfishes
1963	Hawaii	1,351	167	12.4
1964	Hawaii	1,608	342	21.3
	Equator- 10° S	579	268	46.3
	10° - 20° S	216	92	42.6
1965	Hawaii	1,008	193	19.1
	Equator- 10° S	208	60	28.8
	10° - 20° S	537	191	35.6

slightly more numerous north of lat 10° S than between lat 10° and 20° S. In 1965, however, juveniles were more numerous between lat 10° and 20° S than north of lat 10° S. Also they appeared to be more numerous in the South Pacific than near the Hawaiian Islands.

About 12% of the billfish stomachs from Hawaii contained one or more juvenile skipjack tuna, while 19% of the billfish stomachs from the South Pacific contained one or more juveniles. In both areas the largest number of juvenile skipjack tuna per stomach was 11; most of the stomachs from both areas had only one juvenile.

AGE AND GROWTH

The length-frequency distribution of juvenile skipjack tuna from Hawaii and the South Pacific is shown in Figure 5. Near Hawaii the smallest juvenile taken was 5.9 cm SL (standard

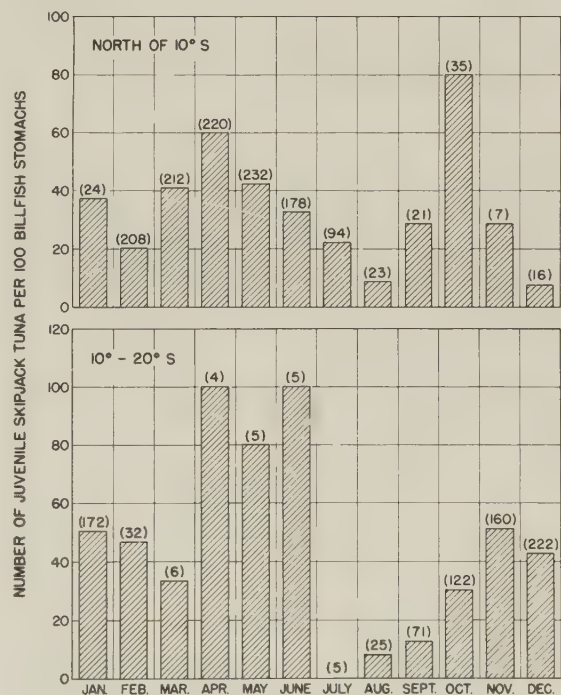


FIGURE 4.—Apparent abundance of juvenile skipjack tuna in the South Pacific. The figures in the parentheses are the number of billfish stomachs that were examined.

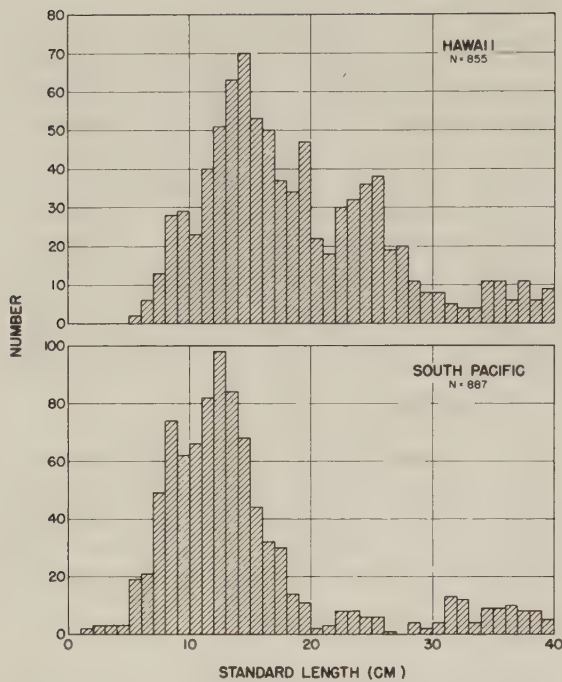


FIGURE 5.—Length-frequency distribution of juvenile skipjack tuna.

length). A prominent mode was located at the 14-14.9 cm size class, a lesser mode at 25-25.9 cm, and a relatively obscure mode was evident between 30 and 40 cm. The length frequencies of juvenile skipjack tuna from the South Pacific were similar to those from Hawaii. The smallest juvenile taken in the South Pacific was 1.6 cm SL. A prominent mode was evident at the 12-12.9 cm length class, a lesser mode between 20 and 30 cm, and a relatively obscure mode was apparent between 30 and 40 cm.

The monthly length-frequency distributions of the juveniles near Hawaii show well-defined modes in the summer and fall (Figure 6). Some of the length distributions have two or more well-defined modes. These modes probably represent progeny from two or more spawnings spaced more or less closely in time. An increase in size of the juveniles, as indicated by a progression of the modes, was evident in the monthly length distributions. In contrast, Higgins (1970) found that modal groups of juvenile

skipjack tuna taken in midwater trawls did not show an increase in length with time.

Because the modal lengths increased with time, an attempt was made to describe the growth of the juveniles. To facilitate the designation of modes, the length data were smoothed by a moving average of three. Modes were not considered in samples that had fewer than 10 fish, nor in length groups containing fewer than 5 fish. The selected modes are given in Figure 7 and Table 3. Using the method of least squares, straight lines were fitted to groupings of modal points to represent the apparent growth of the juveniles. The slope of the lines for 1962, 1964-65, and 1965-66 indicates that juvenile skipjack tuna grow about 2.0 cm per month. The slope of the line for 1963-64 indicates a growth of 1.2 cm per month. These data suggest annual differences in the growth of the juveniles. I believe that an increment of 2 cm per month is the best estimate of the growth rate of juvenile skipjack tuna within the length range covered in this study. Rothschild (1967) used data from tag and recapture experiments and the von Bert-

TABLE 3.—Modes in the length-frequency distributions of juvenile skipjack tuna from Hawaii (from Figure 6).

Year	Month	Standard length (cm)		
1962	August	13.0	19.0	23.5
	September	20.0		
	October	27.0		
	November	22.0		
1963	June	13.5		
	July	13.5		
	August	9.5	15.5	
	September	15.5	22.0	
	November	23.5		
	December	24.0		
1964	April	30.0		
	May	19.0	25.0	
	June	15.5	25.5	
	July	8.5	13.5	
	August	11.0	18.5	
	September	12.5		
	October	14.5	23.5	
	November	23.5		
1965	December	25.0		
	April	35.5		
	May	12.5	26.5	
	June	9.5		
	July	15.5		
	August	10.5	14.5	18.5
1966	October	13.0	19.5	
	November	18.5		
	April	32.5		

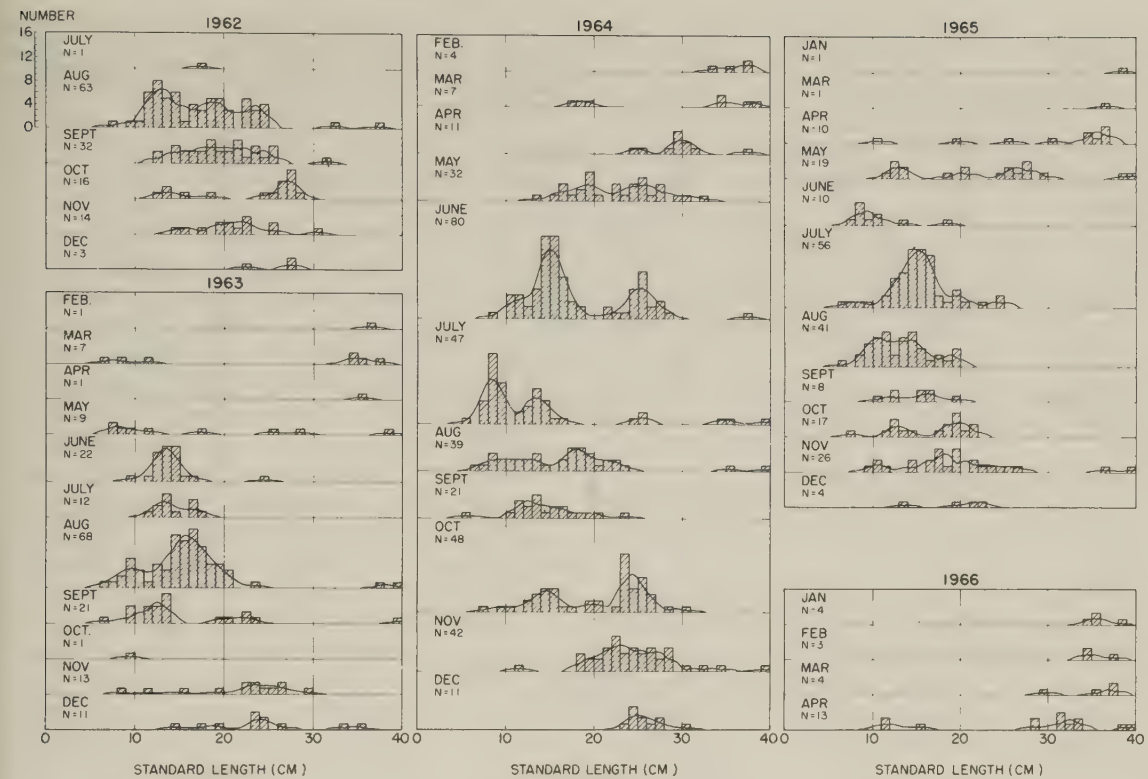


FIGURE 6.—Monthly length-frequency distribution of skipjack tuna from Hawaii.

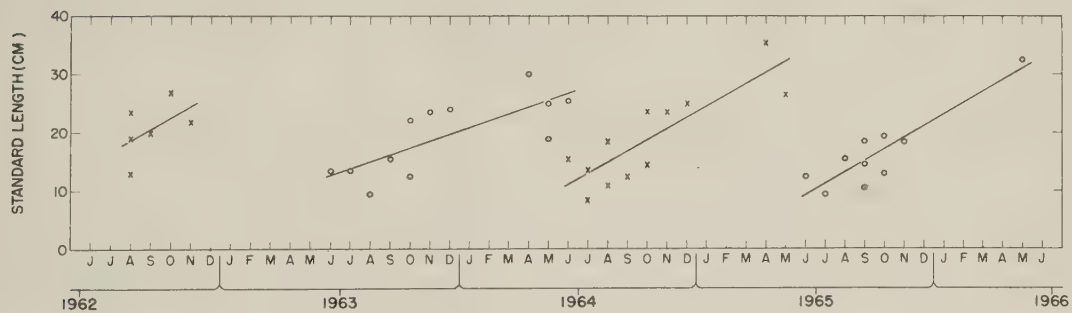


FIGURE 7.—Growth of juvenile skipjack tuna from Hawaii.

alanffy growth function to estimate skipjack tuna growth. He indicated that skipjack tuna between 44 and 72 cm grew about 16.6 cm per year or 1.4 cm per month. The deceleration in growth from 2.0 cm per month for skipjack

tuna between 9 and 40 cm to 1.4 cm per month for fish between 44 and 72 cm is to be expected. It is useful to determine the growth rate of skipjack tuna from hatching to 9 cm SL. These and other data may make it possible to estimate

the age of skipjack tuna at various sizes. The growth rate of a related species, black skipjack, *Euthynnus lineatus*, between 17 and 75 mm SL has been determined (Clemens, 1956). In experiments conducted in shipboard aquaria Clemens showed that black skipjack grew 36.8 mm in 295 hr, which would be about 9 cm in a month. Houde and Richards (1969) reared larval little tunny, *E. alletteratus*, under laboratory conditions and reported similar growth rates. It was recently found that larval skipjack tuna have similar growth rates (personal communication, William J. Richards, Supervisory Zoologist, NMFS Tropical Atlantic Biological Laboratory, Miami, Fla. 33149, August 18, 1970). If larval skipjack tuna grow at the same rate (9 cm per month) for the first month and 2 cm per month for the next 11 months, then skipjack tuna 1 year old would be 31 cm SL.

Brock (1954) assumed that the modal sizes of fish between 40 and 50 cm FL (fork length) that appear in the Hawaiian skipjack tuna fishery during the summer represented 1-year-old fish. Forty to fifty centimeters for 1-year-old fish seems too high. Modal lengths typical of winter skipjack tuna in the Hawaiian fishery are 35, 50, and 70 cm FL (Rothschild, 1965). My data indicate that the 35-cm modal group that appears in the winter represents 1-year-old fish.

SPAWNING

In the Hawaiian area, juvenile skipjack tuna smaller than 10 cm were found during 7 months in 1963 and during 5 months in 1964 and 1965 (Figure 6). This suggests a protracted spawning season and nearly continuous recruitment of juveniles. The catch of larvae indicates that spawning begins in March, peaks in July, and declines sharply in September and October (Matsumoto, 1966).

The Hawaiian skipjack tuna fishery peaks in the summer when the bulk of the catch is composed of "season-size" fish larger than 60 cm (Rothschild, 1965). Larval and juvenile skipjack tuna also are most numerous during the summer, which suggests that the large fish spawn in Hawaiian waters. The presence of

juveniles in the spring and fall, although in lesser numbers, indicates that spawning also takes place then. Rothschild (1965) has hypothesized that at least one subpopulation spawns in Hawaiian waters. The protracted spawning season may indicate that more than one subpopulation spawns here.

In the South Pacific between lat 5° and 20° S, the spawning season is more protracted than in Hawaiian waters. Juveniles smaller than 10 cm SL were taken in almost every month north of lat 10° S. Between lat 10° and 20° S they were taken in all months except March, April, June, and July (Figure 8); the reason for their absence in these months may be inadequacy of sampling. The area south of lat 20° S was somewhat different. Although sampling was sparse, the length-frequency distribution consistently showed few small juveniles, which indicates little or no spawning in this area.

The length-frequency distribution of juvenile skipjack tuna north of lat 10° S and between lat 10° and 20° S indicated a progression of modes (Figure 8). However, a plot of the modal lengths indicated no growth. The cause of this may be a protracted spawning season and inadequacy of samples. Also juveniles appear to migrate southward as they grow. Only small numbers of juveniles larger than 20 cm SL were taken north of lat 20° S, and most of the juveniles taken south of lat 20° S were larger than 20 cm SL.

ACKNOWLEDGMENTS

Thanks are due the research assistants at the NMFS Hawaii Area Fishery Research Center who examined the billfish stomach contents for juvenile skipjack tuna and aided in summarizing part of the data. The portion of the study in the South Pacific would not have been possible without the cooperation of the crews of the many fishing vessels from Japan and the Republic of Korea. I thank William J. Richards, NMFS, Miami, Fla.; Walter T. Pereyra, NMFS, Seattle, Wash.; and Witold L. Klawe, Inter-American Tropical Tuna Commission, La Jolla, Calif. for reviewing the manuscript.

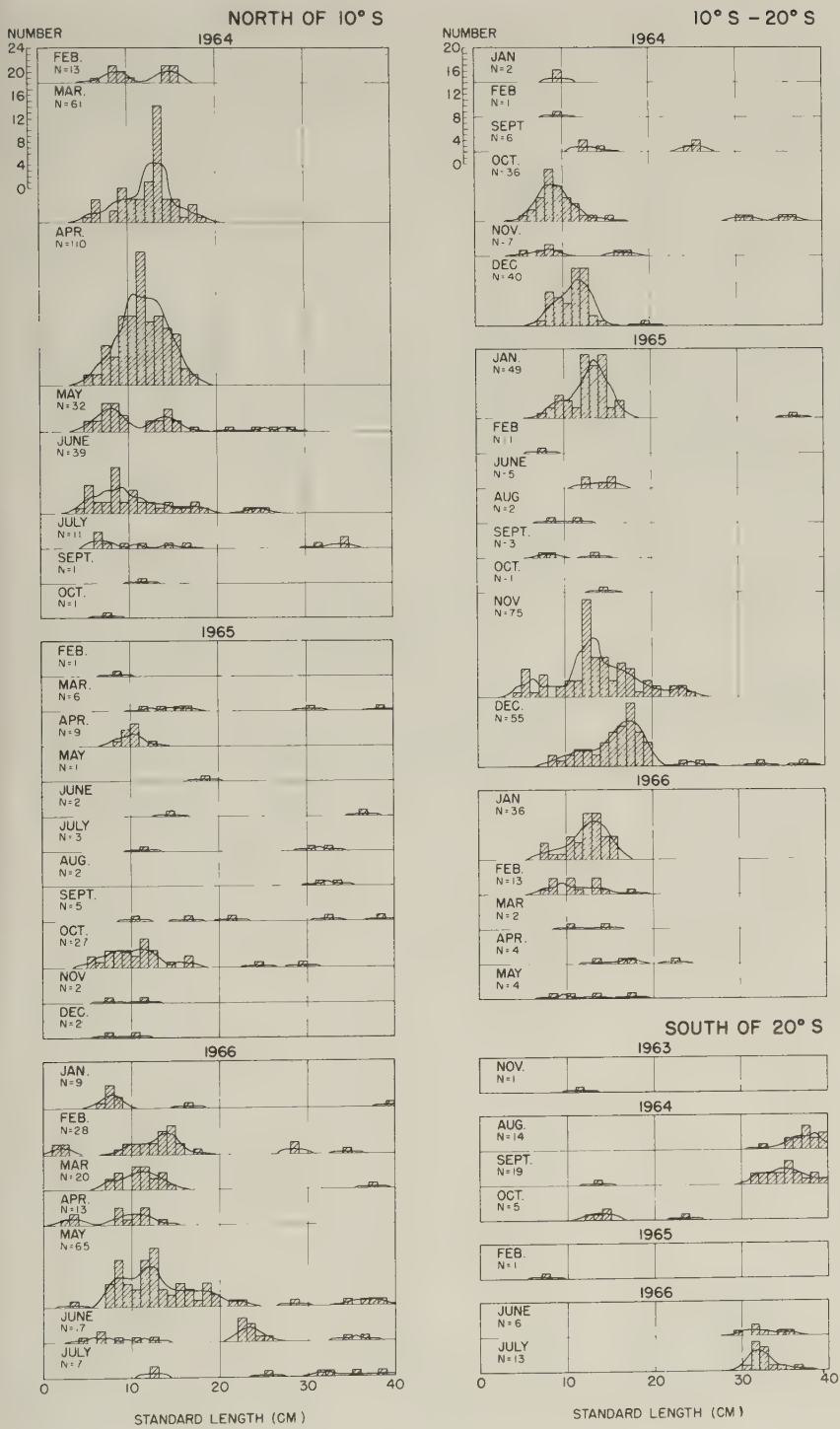


FIGURE 8.—Monthly length-frequency distribution of juvenile skipjack tuna north of lat 10° S, lat 10°-20° S, and south of lat 20° S in the South Pacific.

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DISTRIBUTION OF TUNA LARVAE (PISCES, SCOMBRIDAE) IN THE NORTHWESTERN GULF OF GUINEA AND OFF SIERRA LEONE¹

WILLIAM J. RICHARDS AND DAVID C. SIMMONS²

ABSTRACT

Investigations of tuna larvae distributions in the northwestern Gulf of Guinea and off Sierra Leone were made during February-April 1964, August-October 1964, and February-April 1965. Larvae of the yellowfin tuna, bigeye tuna, skipjack tuna, little tunny, and frigate mackerels were collected and studied. Analyses of the data indicated that larvae of yellowfin tuna and bigeye tuna migrate to the surface during the day, skipjack tuna migrate to the surface during the night, and frigate mackerels do not seem to migrate at any time. Our data for little tunny were inconclusive. All species were widely distributed over the area but larvae of the commercially important tunas—yellowfin, bigeye, and skipjack—were restricted to waters where surface temperatures were higher than 24° C.

The distribution of tunas varies seasonally in the eastern Atlantic Ocean (Richards, 1969). In 1964 and 1965, the Bureau of Commercial Fisheries research vessel *Geronimo* (cruises 3, 4, and 5) collected tuna larvae in the northwestern Gulf of Guinea and off Sierra Leone. These collections were part of extensive investigations intended to relate the spatial and temporal distributions of tunas to the environment. Cruise 3 was in the northwestern Gulf of Guinea between 10 February and 26 April 1964, which is within the winter-spring "warm season" in the Gulf of Guinea, when sea-surface temperatures are higher than during summer and fall. Cruise 4 was in the northwestern Gulf of Guinea between 5 August and 13 October 1964, which is within the summer-fall "cool season" in the Gulf of Guinea, when sea-surface temperatures are lower than during winter and spring. During cruise 5, collections were made in two areas: the northwestern Gulf of Guinea and off Sierra Leone. The northwestern Gulf of Guinea area was generally the same as that covered in cruises 3 and 4 and collections were made from 14 March to 19 April 1965 within the winter-spring "warm season." The area off Sierra

Leone, which is immediately northwest of the areas covered in cruises 3, 4, and part of 5, was studied from 10 February to 2 March 1965 (see Figure 1).

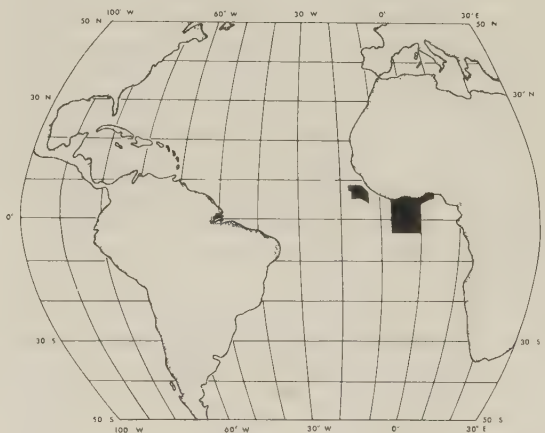


FIGURE 1.—Reference map for the areas studied. The shaded area east of long 10° W was surveyed on *Geronimo* cruises 3, 4, and part of 5; the shaded area west of long 10° W was surveyed on part of cruise 5.

The purposes of this study are to (1) analyze the time the collections were made, (2) describe the distribution of the tuna larvae, and (3) discuss the relations of the tuna larvae to oceanographic features. In addition to the collecting of larvae on each cruise, sightings of surface

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schools of tuna were recorded and several oceanographic features—temperature, salinity, and dissolved oxygen—were measured. The distributions of these oceanographic features were published in a series of atlases (Goulet and Ingham, 1968; Ingham, Goulet, and Brucks, 1968; Brucks, Ingham, and Leming, 1968a, 1968b).

The northwestern Gulf of Guinea is affected by the general meteorological and oceanic conditions of the Gulf of Guinea and by some unique local features. Ingham (1970) concluded that two types of upwelling occur in this region—a seasonal wind-driven upwelling (July through October) and a current-induced upwelling that is present most of the time. The mixed surface layer is rather thin in the coastal area (less than 10 m near the coast, grading to 30 to 40 m offshore) and is influenced by current-induced upwelling, wind-driven upwelling, and advection. Ingham (1970) reported that during the period of *Geronimo* cruises 3, 4, and 5, advection was the most effective of the three factors.

The species collected were the yellowfin tuna, *Thunnus albacares* (Bonnaterre); the bigeye tuna, *Thunnus obesus* Lowe; the bluefin tuna, *Thunnus thynnus* (Linnaeus); the skipjack tuna, *Katsuwonus pelamis* (Linnaeus); the little tunny, *Euthynnus alletteratus* (Rafinesque); and the frigate mackerel, *Auxis* sp. Larvae of the albacore, *Thunnus alalunga* (Bonnaterre) were not collected. Numbers of larvae, their location, and the methods used to collect, sort, identify, and compute the numbers of larvae have been treated by Richards et al. (1969a, 1969b, 1970) for each cruise. Larvae were collected by an ICITA (International Cooperative Investigations of the Tropical Atlantic) 1-m plankton net, towed at the surface.

ANALYSIS OF COLLECTION TIME

The relative apparent abundance of some fish larvae is complicated by diel variations. Oblique plankton collections that sample the entire vertical distribution of a species tend to catch fewer fish larvae during the day (Ahlstrom, 1959), presumably a result of increased net avoidance. In surface collections, such as those taken dur-

ing *Geronimo* 3, 4, and 5, diel vertical migrations also could be an important factor in abundance variations.

We used the Mann-Whitney U test (Siegel, 1956) to determine the probability of equal catches of tuna larvae in day and night surface tows. A ranked test such as this should minimize the effects of patchiness. Tows with local apparent midtimes from 0600 through 1759 hr were designated as day tows and those with local apparent midtimes from 1800 through 0559 hr as night tows. Included in our calculations were all successful tows (those that captured tuna larvae) and unsuccessful tows (those that did not capture tuna larvae), except those unsuccessful tows outside the temperature-salinity ranges of the species (Table 1). These temperature-salinity ranges are a composite from Richards (1969) and the present study and should not be considered absolute. The unsuccessful tows were included because of the implication that larvae were not captured for some reason other than intolerance to temperature or salinity. In calculating the statistics, a correction for the tied (equally ranked) unsuccessful tows was used (Siegel, 1956).

TABLE 1.—Temperature-salinity ranges for larvae of yellowfin tuna, bigeye tuna, skipjack tuna, little tunny, and *Auxis* sp. These data are a composite from Richards (1969) and the present study.

Species	Temperature range	Salinity range
	° C	‰
Yellowfin tuna	23.6-29.7	33.5-36.8
Bigeye tuna	23.6-30.5	31.8-36.4
Skipjack tuna	23.4-29.7	31.4-36.9
Little tunny	22.7-29.3	32.7-35.4
<i>Auxis</i> sp.	21.6-30.5	33.2-35.9

The resulting probabilities (Table 2) indicate that yellowfin and bigeye tunas were collected more often at the surface during the day, and skipjack tuna and little tunny more often at the surface at night. No difference was apparent between day and night tows for *Auxis*.

Also analyzed was whether tuna larvae were better able to dodge the plankton net during the day than at night. The question was considered because we naturally assumed that tuna larvae should be able to see a plankton net more clearly during the day and therefore avoid it more

TABLE 2.—Probabilities of equal catches of larvae in day and night plankton tows.

Species	Number of day tows	Number of night tows	Total number of standardized larvae per number of successful tows		Probability of equal catches
			Day	Night	
Yellowfin tuna	263	194	1701.7/113	645.8/57	<0.01
Bigeye tuna	265	197	409.6/68	186.4/32	=0.02
Skipjack tuna	267	206	33.3/12	364.7/38	<0.01
Little tunny	274	209	303.5/28	362.2/36	=0.03
<i>Auxis</i> sp.	280	218	3637.8/128	2812.3/93	=0.99

easily. We also reasoned that large larvae, being better swimmers than smaller larvae, should have been captured less frequently in day collections than in night collections. Thus, if net avoidance was demonstrable, the lengths of larvae caught during the day should have been smaller.

Percent length frequencies of each species of larvae collected in the day and night were plotted from the following data: Yellowfin tuna, 1,009 day-caught larvae, 340 night-caught larvae (Figure 2); bigeye tuna, 271 day, 84 night (Figure 3); skipjack tuna, 22 day, 197 night (Figure 4); little tunny, 134 day, 72 night (Figure 5); and *Auxis*, 1,636 day, 1,082 night (Figure 6).

The length frequencies of night-caught larvae tended to be skewed more toward the larger sizes than did the day-caught larvae. The bimodal frequency of skipjack tuna captured during the day could have been due to the small sample size. The Mann-Whitney U test (Siegel, 1956) was applied to the frequencies to determine if night-caught larvae were significantly larger than day-caught larvae. Probabilities of less than 0.01 that larvae were the same length were found for yellowfin tuna, little tunny, and *Auxis*; bigeye and skipjack tunas had probabilities of 0.06 and 0.22, respectively. It should be noted also that the largest larvae of every species but bigeye tuna were captured at night. We tentatively conclude that there was greater net avoidance during the day for yellowfin tuna, little tunny, and *Auxis*, but little net avoidance for bigeye and skipjack tunas.

A differential vertical migration on the basis of size also should be considered as a possible explanation for the capture of larger larvae at night. Certain evidence causes us to reject this possibility, however. Ueyanagi (1969) found

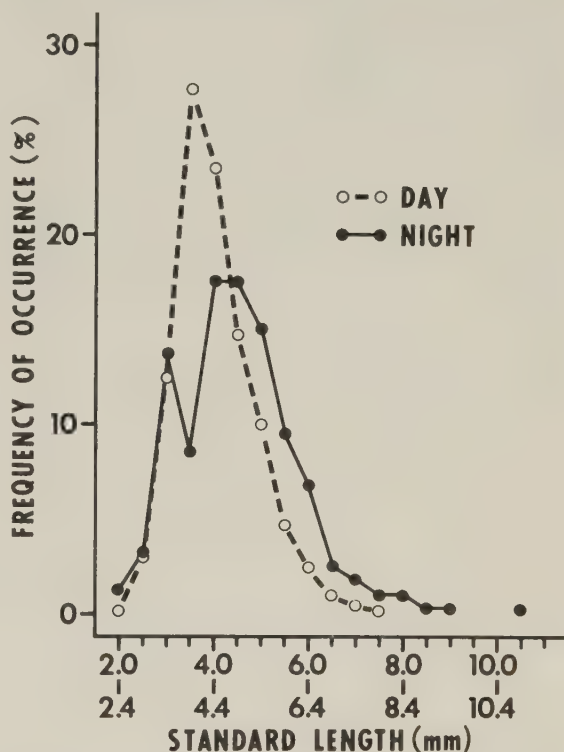


FIGURE 2.—Percent length frequencies of yellowfin tuna larvae captured during the day (broken line, 1,009 specimens) and night (solid line, 340 specimens).

that the size composition of tuna larvae taken in night surface tows resembled the size composition of those taken during both day and night at depth. Smaller larvae were more numerous in catches made at the surface during the day. The implication is that net avoidance of larger larvae is greater at the surface during the day, and there is no indication of a vertical migration of the two size groups in opposition to one another.

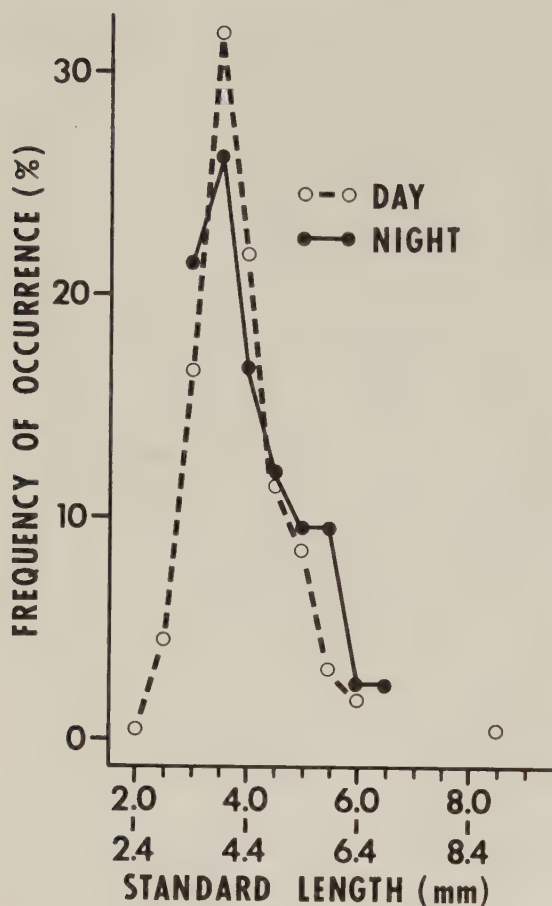


FIGURE 3.—Percent length frequencies of bigeye tuna larvae captured during the day (broken line, 271 specimens) and night (solid line, 84 specimens).

Analyses of our data show that yellowfin tuna larvae were more successfully captured in day tows than at night, even though greater net avoidance during the day was indicated. Had net avoidance been the major factor in day-night differences in abundance, more larvae should have been captured at night. Apparently—since the opposite is indicated—yellowfin tuna larvae migrate to the surface in the day and net avoidance is of minor importance, in terms of numbers collected. Ueyanagi (1964) suggested that istiophorid larvae behave similarly; other workers (Wade, 1951; Strasburg, 1960; Klawe,

1963; Ueyanagi, 1969) found no decisive evidence to show that yellowfin tuna larvae perform a vertical diel migration to the surface.

Our study indicated that bigeye tuna larvae—like those of yellowfin tuna—migrate vertically to the surface in the day, but the probabilities were not as significant ($P = 0.02$ compared with $P < 0.01$ for yellowfin tuna). Net avoidance was negligible for bigeye tuna larvae. Ueyanagi (1969) reported a greater larval occurrence of bigeye tuna at the surface during the day than at night.

Our evidence showed that skipjack tuna larvae migrate vertically to the surface at night and that net avoidance was apparently negligible. A vertical migration to the surface at night also was suggested by Wade (1951) and Strasburg (1960). Ueyanagi (1969) reported a scarcity at the surface during the day, but increased abundance at night.

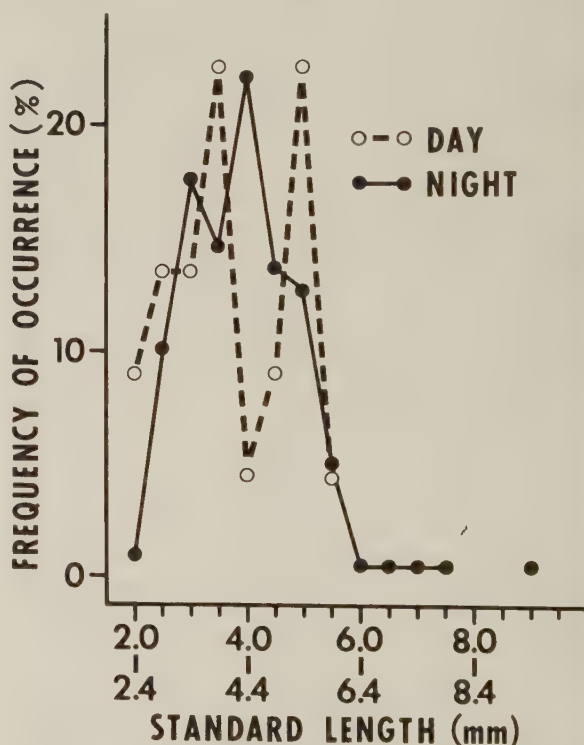
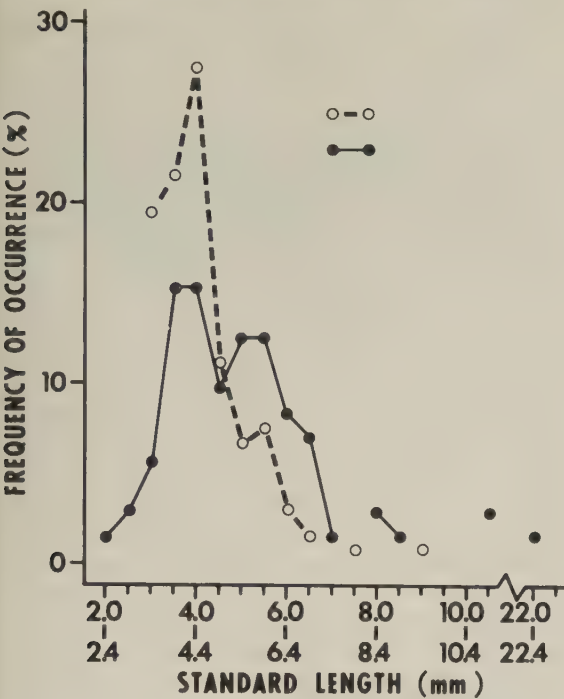


FIGURE 4.—Percent length frequencies of skipjack tuna larvae captured during the day (broken line, 22 specimens) and night (solid line, 197 specimens).



Our night tows caught little tunny larvae more successfully than day tows, but differences were not as pronounced as they were for skipjack tuna larvae ($P = 0.03$ compared with $P < 0.01$ for skipjack tuna larvae). Since a greater ability to dodge the net during the day was indicated, day-night differences could have been caused by migration to the surface at night, net avoidance, or a combination of both. Among larvae of yellowfin tuna, bigeye tuna, skipjack tuna, and *Auxis*, net avoidance was negligible or ineffective in detecting day-night differences in abundance. The higher frequency of night captures of little tunny larvae, therefore, was probably caused primarily by vertical migration to the surface at night. Vertical migration to the surface at night also was suggested for the closely related *Euthynnus yaito* ($=E. affinis$) by Wade (1951).

FIGURE 5.—Percent length frequencies little tunny larvae captured during the day (broken line, 134 specimens) and night (solid line, 72 specimens).

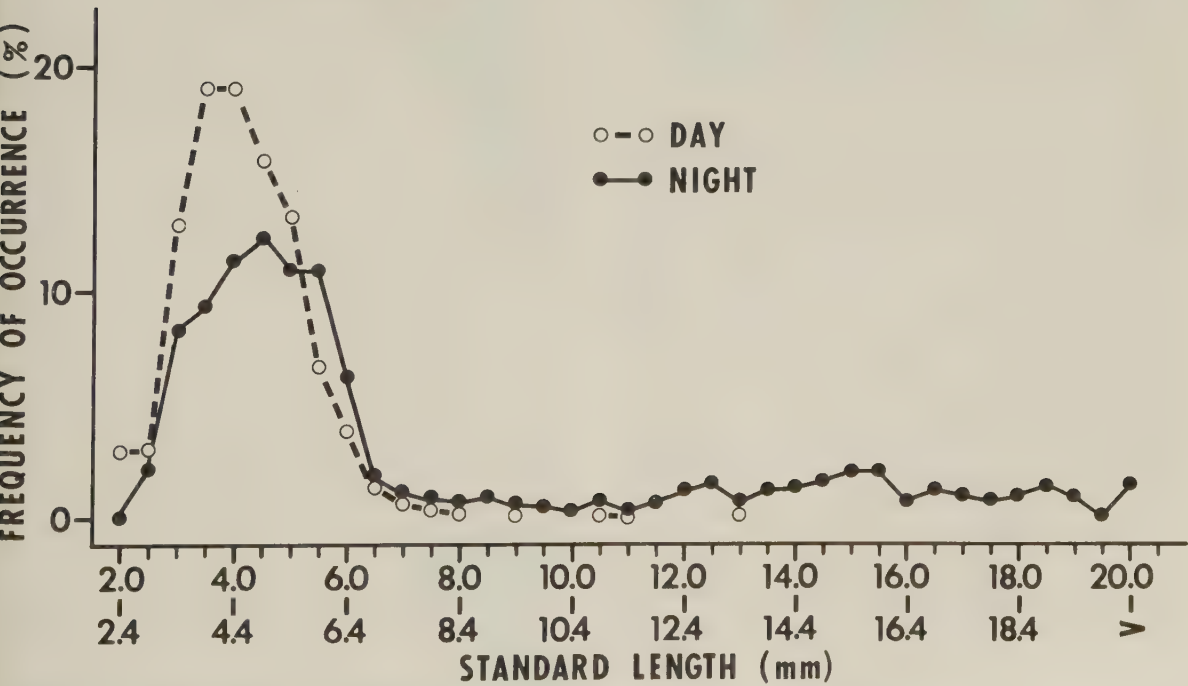


FIGURE 6.—Percent length frequencies of *Auxis* larvae captured during the day (broken line, 1,636 specimens) and night (solid line, 1,082 specimens).

Auxis larvae were equally abundant in day and night tows, indicating that this species does not migrate to the surface. The indication of net avoidance during the day had no detectable effect on apparent abundance, but if *Auxis* larvae were more abundant at the surface during the day and net avoidance had a significant effect on abundance, the same results could be obtained. Larval *Auxis* were almost equally abundant at the surface in day and night collections according to Wade (1951). Strasburg (1960) captured more *Auxis* larvae in 0 to 60 m tows at night and stated that Matsumoto (1958) also captured more specimens in night surface tows. Klawe (1963) reported greater success in catching *Auxis* larvae at night in surface and 300-m oblique tows but not in 140-m oblique tows; he suggested that net avoidance may be primarily responsible for decreased day catches. In a more recent study, Klawe, Pella, and Leet (1970) concluded that *Auxis* larvae did not exhibit a diel vertical movement; they also found no indication of net avoidance.

DISTRIBUTION OF LARVAE

Because all our collections were made by surface tows, it was not possible to directly compare our totals with the number of larvae collected during the Equalant surveys (Richards, 1967, 1969). The two multiship Equalant surveys covered most of the tropical Atlantic Ocean. Equalant I took place at the same time of year as *Geronimo* cruises 3 and 5 ("warm season"), Equalant II corresponded to the time of *Geronimo* cruise 4 ("cool season"). The average number of tuna larvae collected per 1,000 m³ of water strained on each *Geronimo* cruise herein discussed and the average under 1 are (100 m²) of sea surface for Equalant I and Equalant II (Richards, 1969) are shown in Table 3. The average numbers of larvae collected on the *Geronimo* cruises were corrected for diel variations in abundance. This was computed by the following formula:

$$\frac{a/b + a'/b'}{2}$$

where a = total number of standardized day-caught larvae
 a' = total number of standardized night-caught larvae
 b = total number of day tows
 b' = total number of night tows.

The correction was applied to all species except *Auxis* because that species was equally abundant in day and night collections. The averages for *Auxis* were obtained by dividing the total number of standardized larvae by the total number of tows. The Equalant averages were not corrected for diel variations in abundance because most of the collections were oblique and sampled the entire vertical range of all tuna larvae. Calculations for the average number of larvae collected were similar to those used for *Auxis* but were expressed as the number under 1 are of sea surface. In the following separate accounts we report on our detailed findings concerning each species of larval tuna.

TABLE 3.—The average number of tuna larvae collected on *Geronimo* cruises 3, 4, and 5 and the two Equalant surveys.

Species	Geronimo cruise				Equalant survey	
	3	4	5	5	I	II
	Number per 1000 m ³				Number under 1 are	
Yellowfin tuna	11.4	5.2	1.1	1.0	7.82	5.05
Bigeye tuna	2.9	0.9	0.4	0.6	3.00	1.24
Skipjack tuna	2.3	0.3	0.1	0.2	13.71	7.85
Little tunny	3.5	0.8	0.4	0.4	--	--
<i>Auxis</i> sp.	12.6	9.9	6.5	18.8	--	--

¹ 14 March to 19 April 1965 in northwestern Gulf of Guinea.

² 10 February to 2 March 1965 off Sierra Leone.

YELLOWFIN TUNA LARVAE

The distribution of yellowfin tuna larvae in the northwestern Gulf of Guinea is shown in Figure 7. During *Geronimo* cruise 3, yellowfin tuna larvae were common throughout most of the area, averaging 11.4 larvae per 1000 m³ of water strained. During cruise 5 (in the Gulf of Guinea a year later), a smaller area was sampled and an average of 1.1 larvae was collected per 1000 m³ of water strained. During Equalant I, no larvae were found north of about lat 2° N in the same area, in contrast to the distribution found during *Geronimo* cruise 3. We presume

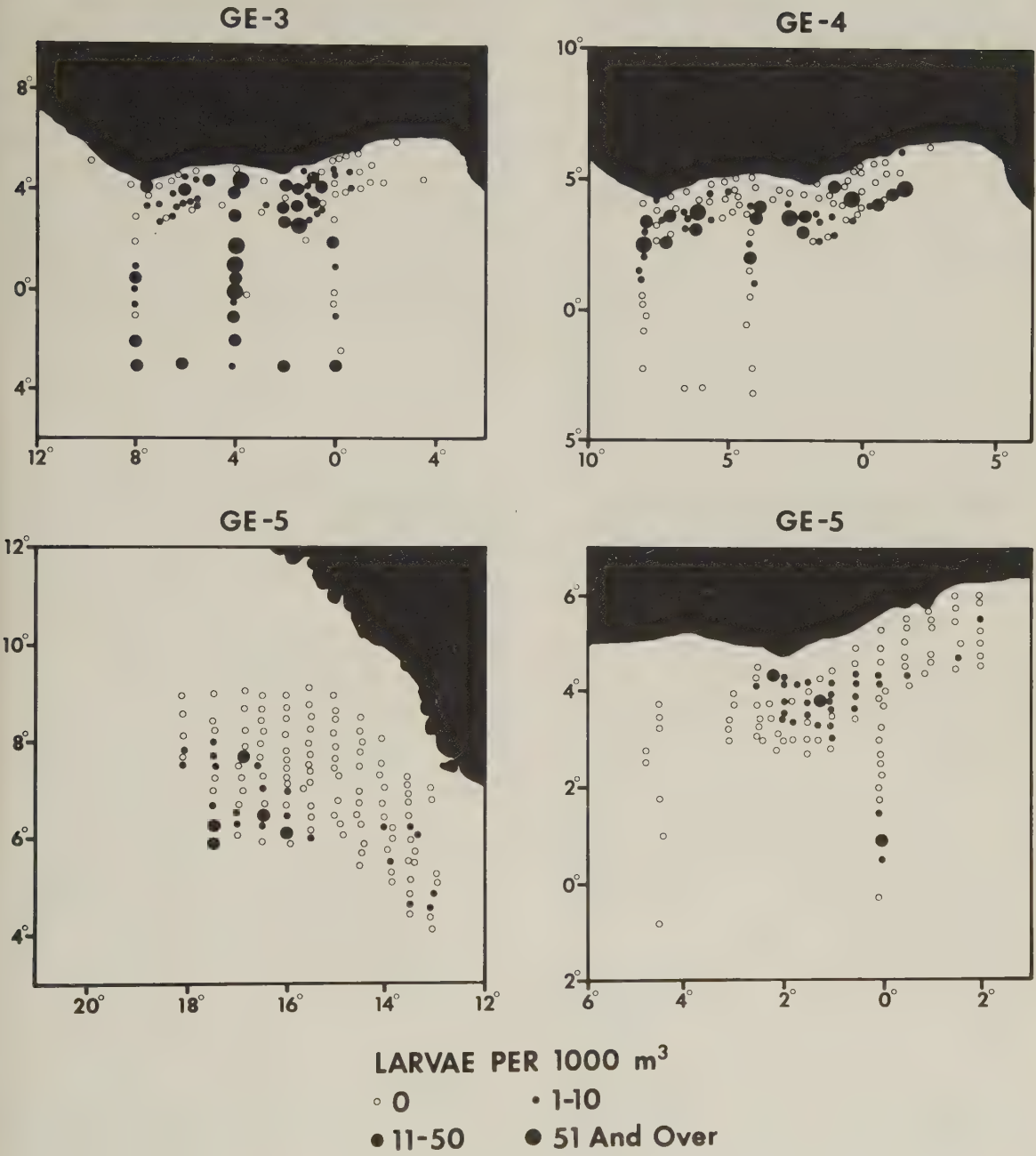


FIGURE 7.—The distribution of yellowfin tuna larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

that the difference was because of increased sampling intensity on *Geronimo* cruise 3. Widespread spawning was seen near the equator, however, on both Equalant I and *Geronimo* cruise 3. An indication of this equatorial spawning is evident in cruise 5 (Figure 7). During *Geronimo* cruise 4, the distribution of larvae was reduced from that seen on cruise 3, averaging 5.2 larvae per 1000 m³ of water strained. Again the situation differed from that found in Equalant II during which almost no larvae were taken, probably because of light sampling.

Richards (1969) found no yellowfin tuna larvae in waters with temperatures lower than 26° C, and indicated that the presence of yellowfin tuna larvae may depend on water temperature. During the *Geronimo* cruises, with one exception, yellowfin tuna larvae were collected in waters warmer than 24° C. Hence, the lower limit of 26° C for surface temperature set for the presence of yellowfin tuna larvae by Richards (1969) should be lowered to 24° C. Surface water temperatures were above 27° C at all stations sampled during cruise 3, and yellowfin tuna larvae were found between 27.9° and 29.7° C. During cruise 5 (also the "warm season"), surface temperatures ranged from 22.5° to 29.9° C but yellowfin tuna larvae were found within a range of 24.9° to 29.5° C. During cruise 4 (the "cool season"), surface temperatures ranged from 19.3° to 25.5° C; yellowfin tuna larvae were found only in water with temperatures higher than 24° C except at one station with a temperature of 23.6° C. During cruises 3, 4, and 5, surface salinity values ranged from 33‰ to 36‰. The yellowfin tuna larvae were rarely encountered when salinity fell below 34‰ but were common between 34‰ and 36‰.

In the area off Sierra Leone, yellowfin tuna larvae were encountered in water temperatures higher than 25° C (Figure 7), the area south of the 25° C isotherm. That area was not covered during the "cool season" by *Geronimo* cruises but did receive minor coverage on Equalants I and II, which resulted in the collection of some tuna larvae, particularly on Equalant II. Water temperatures were 26° C or higher at the Equalant stations where collections were made. Conand (1970) found yellowfin tuna larvae in

waters warmer than 27° C off Senegal.

The Gulf of Guinea and contiguous waters account for much of the Atlantic tuna catch. Beardsley's (1969) discussion of the relation of oceanographic features to adult yellowfin tuna distributions in that area is of interest to the present study. In his summary charts of adult yellowfin tuna distributions, some catch rates are high in areas of cool water where the larvae do not occur, which indicates that an abundance of adults may not indicate abundance of larvae. Surface fishing was carried out by the *Geronimo* during cruises 3, 4, and 5 and it was interesting to note that there was no apparent relation between sightings of surface schools and location of larvae.

BIGEYE TUNA LARVAE

The distribution of bigeye tuna larvae in the northwestern Gulf of Guinea approximated that of yellowfin tuna larvae (Figure 8), but the average number per 1000 m³ of water strained was less than for yellowfin tuna larvae. (A similar pattern was noticed on the Equalant surveys). Off Sierra Leone, the species was collected as often as yellowfin tuna (29 bigeye tuna stations compared with 28 yellowfin tuna stations), but the average number of bigeye tuna larvae collected was less than that of the yellowfin tuna. Larvae of bigeye tuna—like the yellowfin tuna larvae—were collected offshore, south of the 25° C isotherm. The apparent abundance of bigeye tuna larvae, compared with yellowfin tuna larvae, closely resembles that of the adults, as shown in the Japanese Atlantic longline data (Wise³). During the Equalant surveys, 3.0 times more yellowfin tuna larvae than bigeye tuna larvae were captured. In 1963 (the year of Equalants I and II) 3.4 times more yellowfin tuna adults than bigeye tuna adults were captured by Japanese longliners in the same general area (Wise, see footnote 3). During the *Geronimo* surveys 3.9 times more yellowfin tuna larvae than bigeye tuna larvae were captured. In 1964 (the

³ Wise, J. P. 1969. Some basic statistics of the Atlantic tuna fisheries. B.C.F. Tropical Atlantic Biological Laboratory, [Miami, Fla.,] Data Summary No. 8, 14 p. [Processed.]

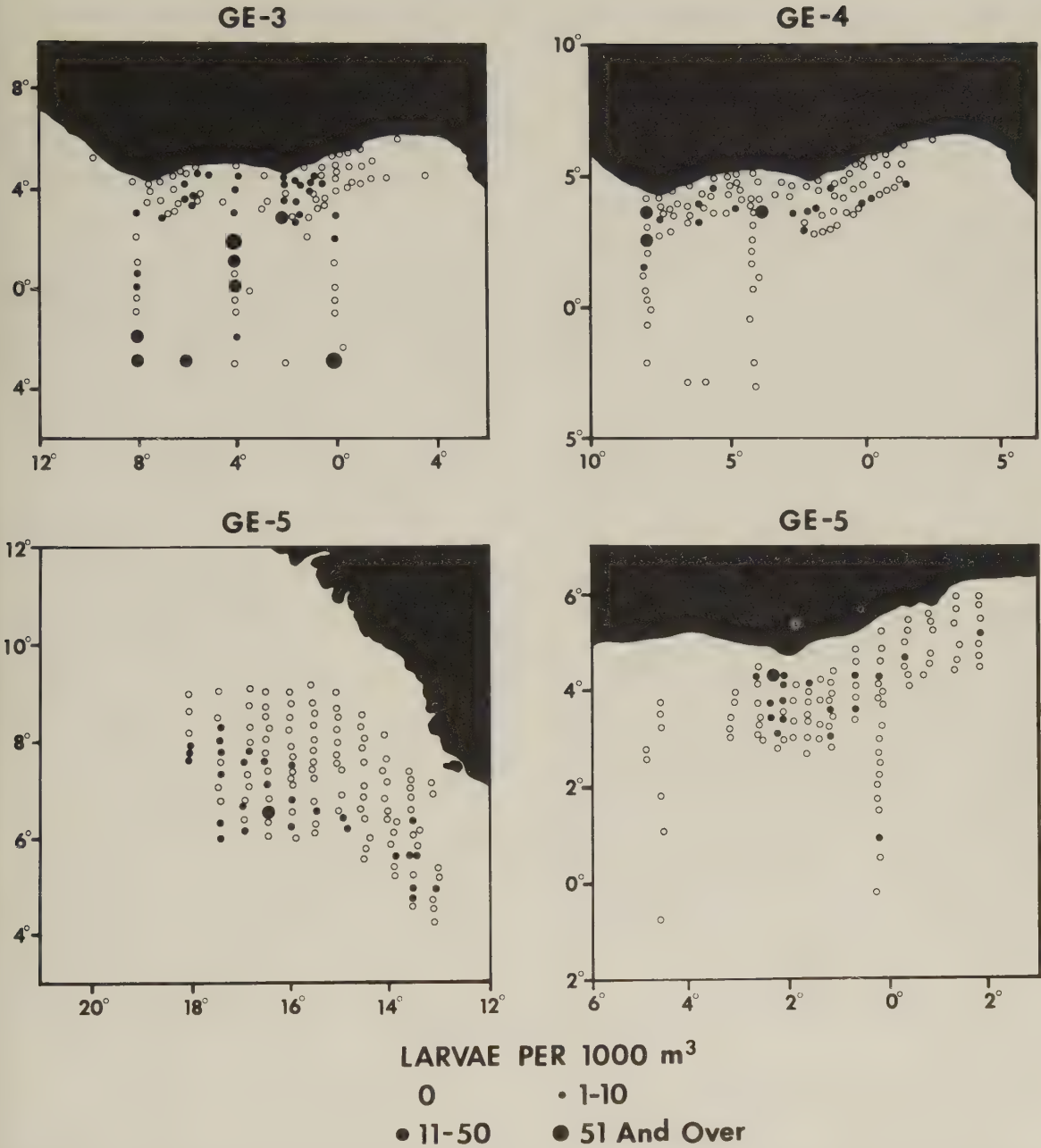


FIGURE 8.—The distribution of bigeye tuna larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

predominant year of the *Geronimo* surveys), 3.7 times more adult yellowfin tuna than adult bigeye tuna were captured by Japanese longliners in the same general area (Wise, see footnote 3).

SKIPJACK TUNA LARVAE

Richards (1969) found that distributions of skipjack tuna larvae differed from those of yellowfin and bigeye tunas, particularly when surface temperature values were below 26° C. Apparently skipjack tuna larvae are able to tolerate lower temperatures than the other two tunas. In the area covered by *Geronimo* cruise 3, distributions of larval skipjack tuna (Figure 9) were similar to those of yellowfin tuna larvae, but fewer were caught. The lesser quantities may have resulted from the sampling method used; surface collections may not adequately sample the species.

On *Geronimo* cruise 3, skipjack tuna larvae were collected in water temperatures that ranged from 27.6° to 29.7° C and salinities from 34.4‰ to 35.5‰. On *Geronimo* cruise 4 (Figure 9) the species was infrequently collected, although larvae had been commonly collected in the same region on Equalant II. Skipjack tuna larvae were found in the warmer water (24.4°-25.8° C) on *Geronimo* cruise 4, which was also true of Equalant II (see Richards, 1969: 298). On *Geronimo* cruise 5 (Figure 9) larval skipjack tunas were taken at only four stations, presumably an artifact of the sampling method. Off Sierra Leone, these larvae were found at only seven stations, perhaps again an artifact of sampling.

LITTLE TUNNY LARVAE

Little tunny larvae were collected during the Equalant surveys but the data have not yet been evaluated. In the northwestern Gulf of Guinea and off Sierra Leone, little tunny larvae were collected during each *Geronimo* cruise (Figure 10). Unlike the other species, they were not collected on the outer transects near the equator. The distribution of the larvae of this species, as it related to temperature and salinity, was

also noticeably different from that of the other tuna species. The temperatures for the larvae ranged from 22.7° to 29.3° C and the salinities from 32.7‰ to 35.4‰. Apparently little tunny larvae can tolerate a wider range of physical conditions than can the larvae of the more oceanic tunas—skipjack, yellowfin, and bigeye.

Auxis sp.

Larvae of *Auxis* (frigate mackerels) are unquestionably the most abundant scombrid larvae found in these tropical waters. This abundance holds true for the eastern Pacific (Klawe, 1963), as well as for the eastern Atlantic (Figure 11). (We are aware that *Auxis* may be two species, but as yet methods for distinguishing their larvae have not been satisfactorily developed.) In the northwestern Gulf of Guinea, *Auxis* larvae were collected mostly nearshore, though a few specimens were found offshore. *Auxis* was the only species widely distributed off Sierra Leone. One reason for its abundance may be the wide tolerance of the larvae for temperature and salinity—*Auxis* larvae were found in water with temperatures as low as 21.6° C and as high as 30.5° C, the widest temperature range found for any tuna larvae we studied. The salinity range of the species was 33.2‰ to 35.9‰.

ACKNOWLEDGMENTS

This manuscript was reviewed by Elbert H. Ahlstrom, National Marine Fisheries Service, La Jolla, Calif.; Witold L. Klawe, Inter-American Tropical Tuna Commission, La Jolla, Calif.; and Walter M. Matsumoto, National Marine Fisheries Service, Honolulu, Hawaii. We appreciate their efforts in our behalf.

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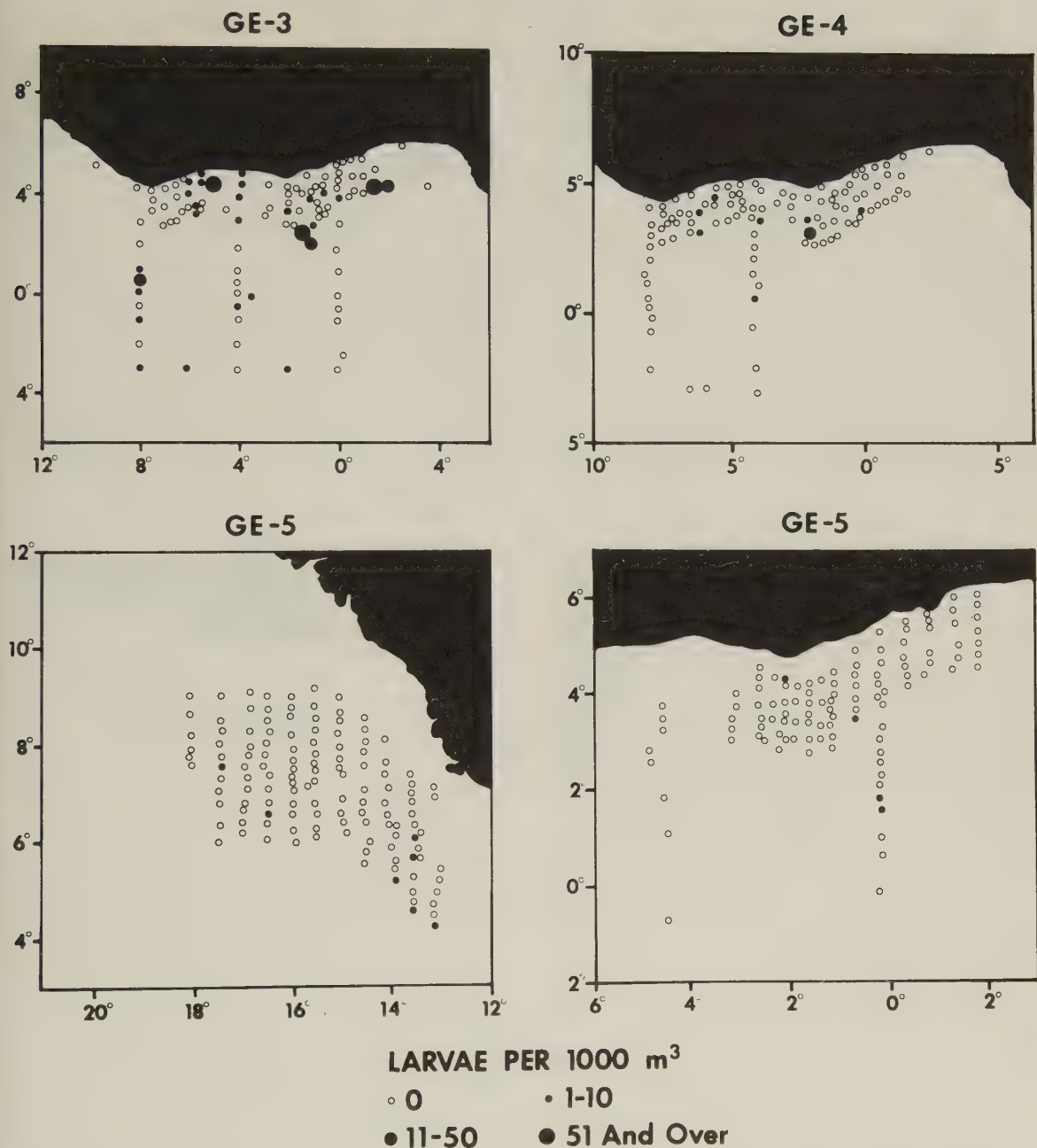


FIGURE 9.—The distribution of skipjack tuna larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

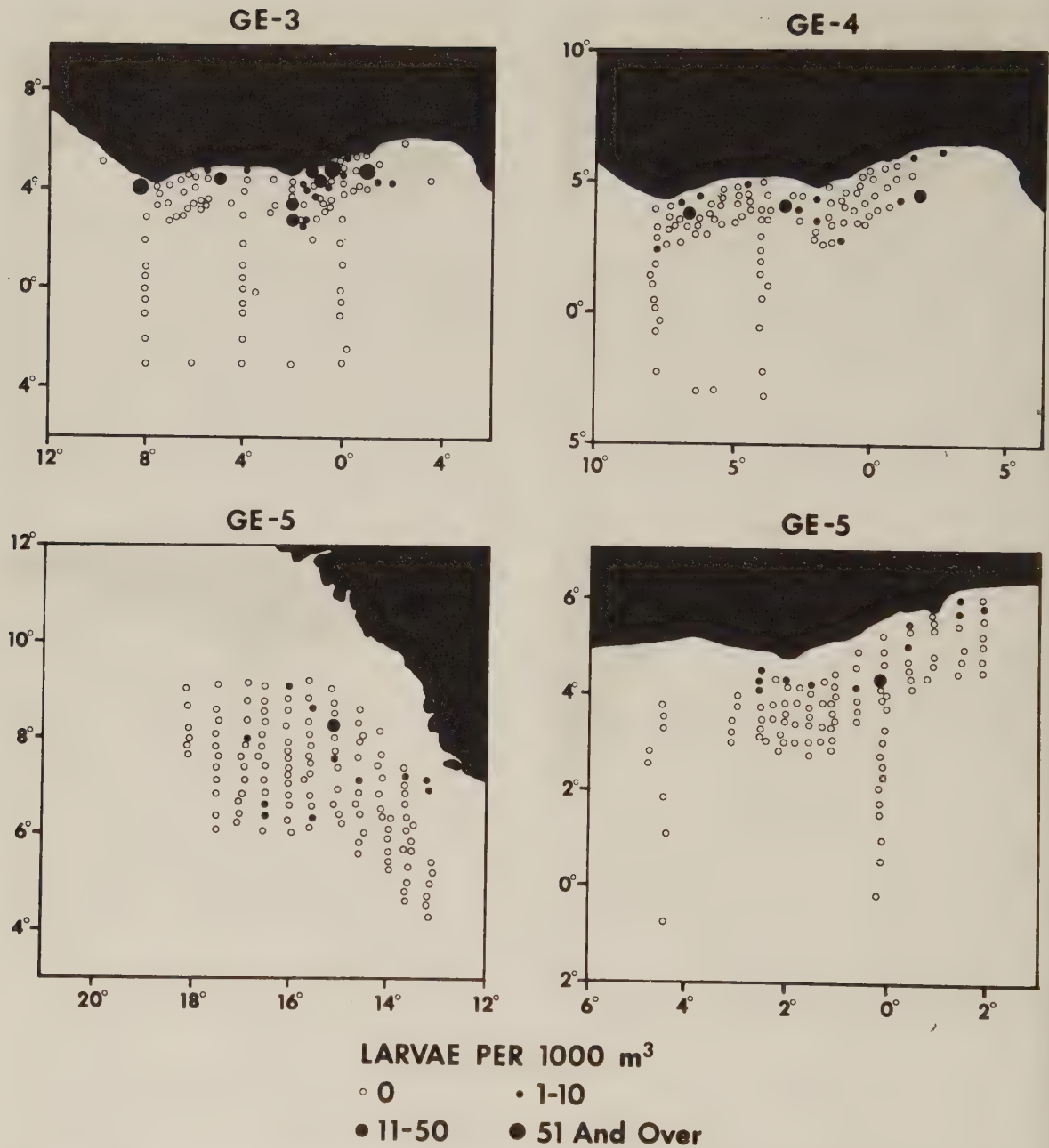


FIGURE 10.—The distribution of little tunny larvae in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

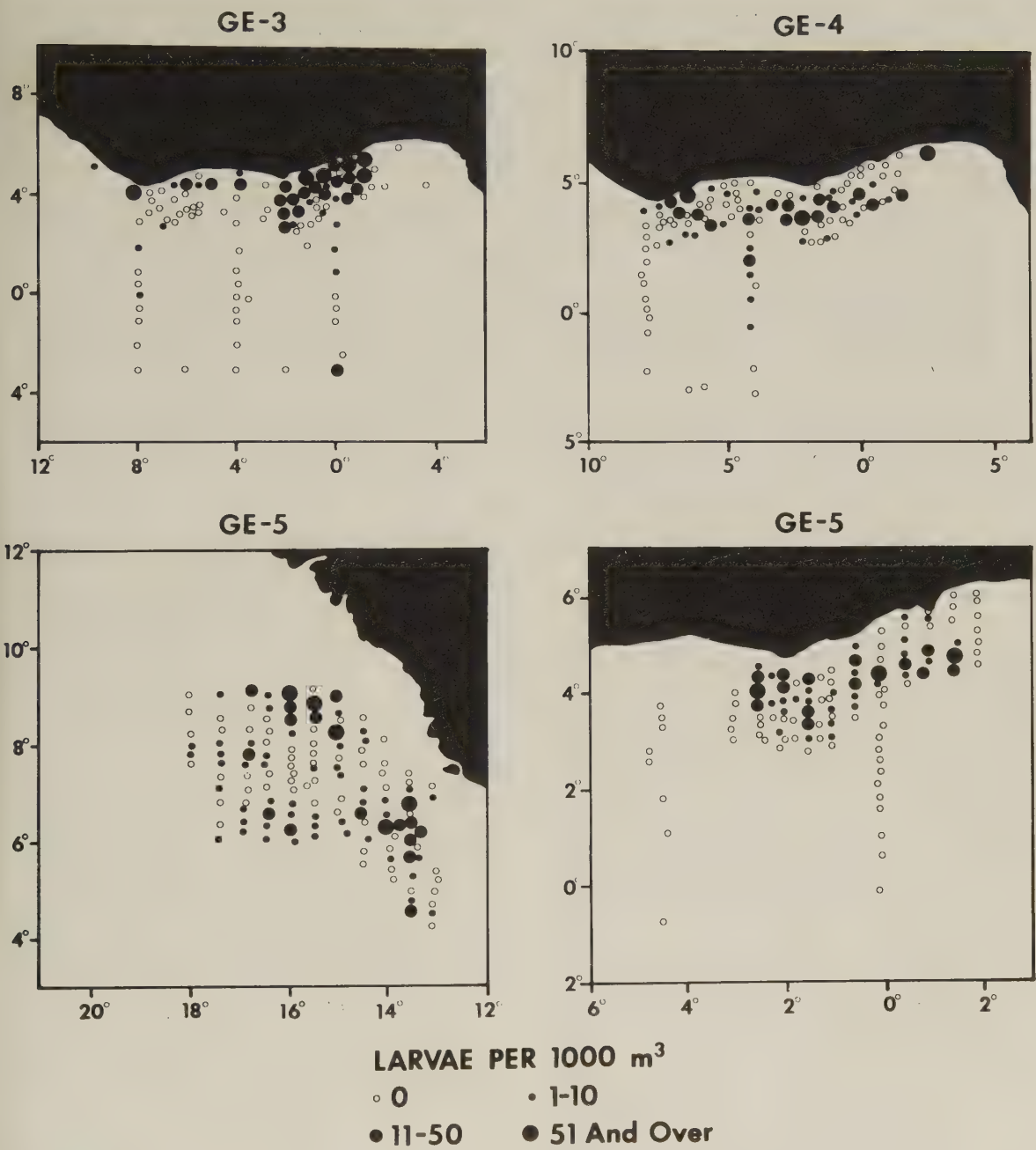


FIGURE 11.—The distribution of frigate mackerel larvae, *Auxis* sp., in the northwestern Gulf of Guinea based on collections during *Geronimo* cruises 3 (10 February to 26 April 1964), 4 (5 August to 13 October 1964), 5 (14 March to 19 April 1965), and cruise 5 off Sierra Leone (10 February to 2 March 1965).

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RANDOM VARIABILITY AND PARAMETER ESTIMATION FOR THE GENERALIZED PRODUCTION MODEL¹

WILLIAM W. FOX, JR.²

ABSTRACT

Three alternative statistical models are proposed for estimating the parameters of the generalized production model by the method of least squares. A stochastic representation of the generalized production model is constructed and simulation (or the Monte Carlo Method) is employed to infer the effects of random variability on the variation in catch. The use of residuals examination for selecting the appropriate statistical model for least-squares estimation of the generalized production model parameters is demonstrated for the yellowfin tuna fishery in the eastern tropical Pacific Ocean. In both the simulation and actual fishery, statistical Model 3—assuming catch residual variance is proportional to the catch squared—best fulfills the assumptions of least-squares theory and should, therefore, provide the best least-square parameter estimates.

Mathematical models are powerful tools which are being used increasingly in resource management. A knowledge of mathematics allows a resource manager to construct from gathered data a representation of the real system and, coupled with statistical theory, allows estimation of the parameters of his model. Then, as is impossible in the real system, a manager may experiment on his model and derive outcomes which aid decisions about management of the real system. Results of model experimentation usually depend greatly on the formulation of the model and to some degree on the accuracy of the parameter estimates. Often precise statistical parameter estimation lags behind mathematical formulation, primarily because many mathematical models are robust, i.e., decisions are independent of parameter accuracy. This is one reason for the development of deterministic rather than stochastic models. However, it seems that it is always desirable to obtain the best possible parameter estimates from the data at hand.

A simple case of Bernoulli's equation has been suggested as a model for the growth of an organism by Richards (1959), Chapman (1961), and Taylor (1962)

$$dx/dt = Hx_t^m - Kx_t \quad (1)$$

where x_t represents either weight or length at time t , and H , K , and m are parameters which may be given some physiological significance. Recently equation (1) has been advanced independently by Chapman (1967) and Pella and Tomlinson (1969) as a simple model for assessing the relation between exploitation and yield (or catch) from a living resource

$$dP/dt = HP_t^m - KP_t - qfP_t \quad \text{for } m < 1 \quad (2)$$

$$dP/dt = -HP_t^m + KP_t - qfP_t \quad \text{for } m > 1$$

where P_t is the population size (biomass or numbers), f is the amount of fishing effort, q is the coefficient of catchability, and H , K , and m are parameters. It is assumed that f is constant over the time period that equation (2) is used. Therefore, $qf = F$, the instantaneous fishing mortality coefficient, and $qfP_t = C_t$ the catch. Equation (2), referred to herein as the generalized production model after Pella and Tomlinson (1969), includes the logistic model used by

¹ Quantitative Science Paper No. 16. A series prepared under the general sponsorship of the *Quantitative Ecology and Natural Resource Management Program* supported by Ford Foundation Grant Number 68-183.

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Graham (1935), Schaefer (1954, 1957), and others when $m = 2$, and the exponential model discussed by Fox (1970) if the limit is taken as $m \rightarrow 1$.

This type of production modeling is a stock assessment approach which has extreme mathematical and data requirement simplicity. Therein lies its primary virtue; for example, equation (2) contains only four parameters whereas the simplest Beverton and Holt (1957) type of model providing the same relation contains at least nine parameters. Estimation of the parameters of equation (2) requires only catch and fishing effort data while at the very least, the Beverton and Holt approach additionally requires age structure information. Discussion of the different assumptions for implementing each approach can be found in Schaefer and Beverton (1963). The generalized production model provides for a wide variety of shapes for the production curve and thus coupled with its mathematical simplicity represents an important tool for successfully managing exploitation.

Procedures for estimating the parameters of production models can be found in Schaefer (1954, 1957), Ricker (1958), Chapman, Myhre and Southward (1962), Gulland (1969), and Pella and Tomlinson (1969). However, it appears that in all cases, except Schaefer (1957), random variation about the deterministic predictions of the production model has been largely ignored in choosing a *statistical* model for estimating the parameters. Perhaps this is because of the apparent formidable nature of such variation. On the other hand, such variation may often be approximated in a simple manner to allow better estimates of the parameters than if ignored altogether. It is conceded that the generalized production model is at the very best only a good approximation of the actual biological dynamics, but this should not imply that better parameter estimates are unwarranted, unless its prime virtue of mathematical simplicity is compromised in the course of such action.

Several statistical models for estimating the parameters of mathematical models of biological relationships have been discussed variously by Zar (1968), Glass (1969), Hafley (1969), and

Pienaar and Thomson (1969). While to the nonstatistician these papers may bear a strong resemblance to quibbling over apparent minor differences of results in the face of large data variability, the improper statistical model can lead to misleading conclusions or to significant errors, as several of the above authors demonstrated. Statistical models differ on the assumption about the manner in which variation or error enters the deterministic biological model. The technique employed by Pienaar and Thomson (1969) to assess fulfillment of the assumptions about variation is the graphing and examination of residuals, the differences between the observed data and those predicted by the model. Extensive discussion on the examination and analysis of residuals can be found in Anscombe (1961), Anscombe and Tukey (1963), and Draper and Smith (1966).

This paper presents a discussion of the nature of simple random variability and its relation to estimating the parameters of the generalized production model. An illustration of residuals examination in selecting the appropriate statistical model for the parameter estimating technique of Pella and Tomlinson (1969) is included. Data from the fishery for yellowfin tuna, *Thunnus albacares*, in the eastern tropical Pacific Ocean were utilized in the illustration.

STATISTICAL MODELS

Schaefer (1957) recognized that the production model is not deterministic and represented environmentally induced variation as an additive term consisting of a random variable η multiplied by population size. In terms of the generalized production model

$$dP/dt = KP_t - HP_t^m - qfP_t + \eta P_t. \quad (3)$$

His parameter-estimating procedure used a finite difference approximation of equation (3) divided through by P_t for the case when $m = 2$. By summing over many time periods the effects of variation are eliminated since the expected value (or mean) of η is zero. Schaefer's formulation of the error term, while reasonable and convenient for his estimating technique, pro-

duces a complex statistical model on integrating equation (3). Therefore, his statistical model was given no further consideration.

Pella and Tomlinson (1969) also mentioned that the generalized production model is not deterministic. They pointed out several sources of error in Schaefer's finite difference approximation of population change and estimation procedure, and advanced a "least-squares" searching procedure as an alternative. In doing so, however, apparently no consideration was given to statistical implications of their technique. The Pella-Tomlinson procedure integrates equation (2) over the time period during which the fishing effort is assumed constant, Δt , to give

$$\hat{P}_t = \left[\frac{H}{K(\pm) qf} - \left(\frac{H}{K(\pm) qf} - P_0^{1-m} \right) \times e^{\mp(K(\pm) qf) (1-m) t} \right]^{\frac{1}{1-m}} \quad (4)$$

where P_0 is the population size at the beginning of the time period, and the upper signs applying when $m < 1$ and the lower when $m > 1$. Starting with initial guesses of the parameter values, an estimated catch history, $\{\hat{C}_i\}$ where $i = 1 \dots n$ time periods, is calculated from the known fishing effort history, $\{f_i\}$, by the formula

$$\hat{C}_i = qf_i \cdot \sum_{j=1}^N \frac{1}{2} (\hat{P}_{i,j} + \hat{P}_{i,j+1}) \cdot \Delta t_i / N \quad (5)$$

where $\hat{P}_{i,j}$ are found from equation (4) over $j = 1 \dots N$ subintervals of each time interval i . The fitting criterion, S , is computed from the known catch history, $\{C_i\}$, of n time periods as

$$S = \sum_{i=1}^n (C_i - \hat{C}_i)^2 = \sum_{i=1}^n \epsilon_i^2 \quad (6)$$

where the ϵ_i are residuals. The initial parameter guesses are then modified in a searching routine with their computer program GENPROD until those parameter values which minimize S are located.

The statistic S is a "least-squares" criterion. For the parameters of a nonlinear model which minimize S to be the best least-squares estimates, the residuals, ϵ_i , must: 1) be independent, 2)

have an expected value (or mean) of zero, and 3) have constant variance (i.e., not correlated with t , $\hat{C}_i$, or f_i).³ Consequently, the proper statistical model for the Pella-Tomlinson fitting technique must both fulfill the three assumptions and be biologically rational. It is also important that the statistical model be simple, i.e., one which requires no additional parameters to be estimated.

Ignoring for the moment that equation (5) is an approximation, the choice of equation (6) as the least-squares estimate criterion tacitly assumes

$$C_i = \hat{C}_i + \epsilon_i \quad (7)$$

giving

$$\bar{P}_i = \hat{P}_i + (1/qf_i) \cdot \epsilon_i \quad (8)$$

where $\bar{P} = \int_0^1 P dt$ for ease of notation. Equation (7), referred to hereafter as statistical

Model 0, is biologically tantamount to assuming random variation in population size approaches being infinitely great in an unexploited population. This denies the concept of an environmentally limited maximum population size or "carrying capacity" which is usually a foundation of the production model. Therefore, Model 0 assumed by Pella and Tomlinson is intrinsically unattractive even though it may be a reasonable approximation at intermediate exploited population levels.

There are three simple statistical models (among many) which are commonly assumed, biologically reasonable, and involve calculating S as a weighted sum of squares or from transformed data.

Model 1. Additive Error

$$\bar{P}_i = \hat{P}_i + \epsilon_{1i} \quad (9)$$

so

$$C_i = \hat{C}_i + (qf_i) \cdot \epsilon_{1i} \quad (10)$$

³ Additionally, if the ϵ_i are normally distributed then it can be shown that the least-squares estimates are also the maximum likelihood estimates which have minimum variance as the number of data grows large—hence are global best estimates (e.g., see Draper and Smith, 1966).

giving

$$S_1 = \sum_{i=1}^n [(C_i - \hat{C}_i)/f_i]^2 \quad (11)$$

as the appropriate criterion to be minimized.

Model 2. Multiplicative Error

$$\bar{P}_i = \hat{P}_i \cdot \epsilon_{2i} \quad (12)$$

so

$$C_i = \hat{C}_i \cdot \epsilon_{2i} \quad (13)$$

or

$$\ln C_i = \ln \hat{C}_i + \ln \epsilon_{2i} \quad (14)$$

giving

$$S_2 = \sum_{i=1}^n (\ln C_i - \ln \hat{C}_i)^2 \quad (15)$$

as the appropriate criterion to be minimized.

Model 3. Additive Proportional Error

$$\bar{P}_i = \hat{P}_i + \hat{P}_i \cdot \epsilon_{3i} \quad (16)$$

so

$$C_i = \hat{C}_i + \hat{C}_i \cdot \epsilon_{3i} \quad (17)$$

giving

$$S_3 = \sum_{i=1}^n [(C_i - \hat{C}_i)/\hat{C}_i]^2 \quad (18)$$

as the appropriate criterion to be minimized.

Model 1 assumes constant variation at all population levels. This is perhaps the least biologically reasonable of the three suggested alternative statistical models since it is easier to conceive that under equilibrium conditions a population will fluctuate more radically near its environmentally limited maximum size than at smaller sizes under constant exploitation. Model 1 is usually employed as a statistical model when variation is expected to arise from experimental or measurement error. Assuming adequate statistics of catch and fishing effort exist, it is more likely that variation will arise from environmental influences on the parameters of the model. Models 2 and 3 assume that variation in popu-

lation size decreases with population size and that variation in catch increases with the size of the catch. Models 2 and 3 approximate the stochastic representation of equation (2) suggested by Pella and Tomlinson [their equation (14)]

$$\begin{aligned} dP/dt = & \eta_1 [(\pm)HP_i^m (\mp) KP_i] \\ & - \eta_2 qfP_i \end{aligned} \quad (19)$$

where η_1 and η_2 are continuous random variables.

Other statistical models obviously could be constructed, such as

$$\bar{P}_i = \hat{P}_i + \hat{P}_i^c \cdot \epsilon_i \quad (20)$$

where c could assume any value—Models 1 and 3 are actually special cases with $c = 0$ or 1 respectively. However, this would introduce another parameter to be estimated. The four previously described statistical models will suffice.

Returning to the point that equation (5) is a numerical approximation of integration, equations (7), (10), (13), (14), and (17) are not strictly true for the Pella-Tomlinson procedure. Accurate representations would include an additional error term due to linear approximation. However, as provided for, the linear approximation error may be reduced by increasing the value of N in equation (5). As will be demonstrated later, this error is very small in relation to the magnitude of the ϵ_i even at small values of N . The choice of N , on the other hand, can be critical to obtaining good estimates of several parameters.

We now have three alternative statistical models which fulfill the goals of simplicity and biological rationality to various degrees. It remains to be determined which of them fulfills the assumptions of least-squares theory for obtaining the best parameter estimates.

STOCHASTIC SIMULATION

An analytical solution for the appropriate statistical model is not possible since the actual causes of variability and the relationships to their effects on the generalized production model

are unknown. However, a commonly used approach, simulation (or the Monte Carlo method), may be employed to infer probable effects of variability and lead to selection of the "best" statistical model. This simulation study consisted of constructing a stochastic (or probabilistic) analogue of the generalized production model and then simulating the catches at various levels of constant fishing effort. Inferences will be drawn about the propriety of all four statistical models from residual variation produced in the catches. Also, the sensitivity of catch residual variation to parameter variation will be demonstrated.

The generalized production model can be written in a form that is more easily discussed biologically

$$\frac{dP}{dt} = P_t K [(P_\infty^{m-1} - P_t^{m-1}) / P_\infty^{m-1}] - qfP_t \quad (21)$$

The signs (+ or -) are set for convenience assuming $m > 1$. The usual biological interpretation of the constants is as follows: K is "the intrinsic rate of natural increase", $P_\infty = (K/H)^{1/(m-1)}$ is the asymptotic environmentally limited maximum population size or "carrying capacity", and m is the determinant of the proportion of P_∞ at which the maximum rate of production occurs. The stochastic analogue of equation (21) is

$$\frac{dP}{dt} = P_t \kappa [(\pi^{\mu-1} - P_t^{\mu-1}) / \pi^{\mu-1}] - \gamma f P_t \quad (22)$$

where $\{\kappa, \pi, \mu, \gamma\}$ are stochastic variables with expected values (or means) $\{K, P_\infty, m, q\}$ respectively, and distributions and variances to be specified. The parameters of equation (21) were considered to be stochastic variables since they are actually average conditions determined by many environmental inter-relationships.

The distributions and variances of the stochastic variables are unknown as are their expected values to be estimated from the fishery data. Some broad inferences about the distributions can be made, however, from biological and mathematical implications of the production model. The "intrinsic rate of natural increase",

κ , was assumed to be approximately normally distributed $[\sim N(K, \sigma^2_1)]$, because K is the resultant rate of a linear combination of rates—birth rate—death rate (P in numbers), or birth rate + growth rate—death rate (P in biomass)—so may be either positive or negative at any given time. Negative values for π and γ are biologically and physically meaningless so they were assumed to be approximately log-normally distributed $[\sim \log N(P_\infty, \sigma^2_2)$ and $\sim \log N(q, \sigma^2_3)$ respectively]. The integrated forms of equations (2), (21), or (22) do not exist for $m = 1$; therefore μ was assumed to be given by $[1 + (m - 1)\xi]$ where ξ was assumed to be approximately log-normally distributed with a mean of one $[\sim \log N(1, \sigma^2_4)]$. This resulted in μ having a mean of m with a range of minus infinity to one, or one to plus infinity, depending on whether m is less or greater than one.

Integrating equation (22) from P_0 to P_t yields

$$\hat{P}_t = \left\{ \pi^{1-\mu} \left(\frac{\kappa}{\kappa - \gamma f} \right) - \left[\pi^{1-\mu} \left(\frac{\kappa}{\kappa - \gamma f} \right) - P_0^{1-\mu} \right] e^{(\kappa - \gamma f)(1-\mu)t} \right\}^{\frac{1}{1-\mu}}; \quad (23)$$

this is the stochastic analogue of equation (4). Expected values and arbitrary variances ($\sigma^2_1, \sigma^2_2, \sigma^2_3, \sigma^2_4$) were chosen to allow:

Stochastic variable	Expected value	Approximate 99% range
κ	5.60	5.30-5.90
μ	2.00	1.95-2.06
π (10^8)	1.40	1.07-1.83
γ (10^{-5})	7.00	6.63-7.39

The expected values were rounded approximate values obtained in the example following this section. In the same manner as the previously described Pella-Tomlinson technique, equations (5) and (23) were used to simulate a 48-year catch history at each of 13 levels of fishing effort. The continuous stochastic variable case was approximated by setting $N = 10$ in equation (5). At each iteration, the stochastic variables $\{\kappa, \pi, \mu, \gamma\}$ were drawn at random from their respective probability distributions, produced

with a random number generator by the multiplicative congruential method (subroutine RAND, University of Washington Computer Center). The variances and means of residuals and log-residuals were calculated at each fishing effort level.

The results of the simulation trials are given in Table 1. It was obvious from the formulation of equation (23) that Model 0—assuming constant residual variance—was inappropriate, the simulation trials add confirmation. Model 1—assuming residual standard deviation proportional to fishing effort—is also rejected over any moderate range of fishing effort. A close approximation, however, is obtained for $f \leq 22,000$.

Model 2—assuming constant log-residual variance—appears to be valid up to $58,000 \leq f < 65,000$, where a trend of increasing variance begins. The hypothesis of common log-residual variance for $f \leq 65,000$ was tested by Bartlett's t -test (Snedecor and Cochran, 1967). The result is not significant (uncorrected $\chi^2 = 7.72$, 9 df, $\text{Pr} > 0.50$). Including the log-residual variance for $f = 70,000$, however, significance is approached (corrected $\chi^2 = 16.35$, 10 df, $\text{Pr} < 0.10$).

Model 3—assuming residual standard deviation proportional to catch—fulfills the assumption about as well as Model 2. The proportional relationship between the residuals standard deviations and deterministic catch (Figure 1) appears to be different between catches given by fishing effort below and above that which pro-

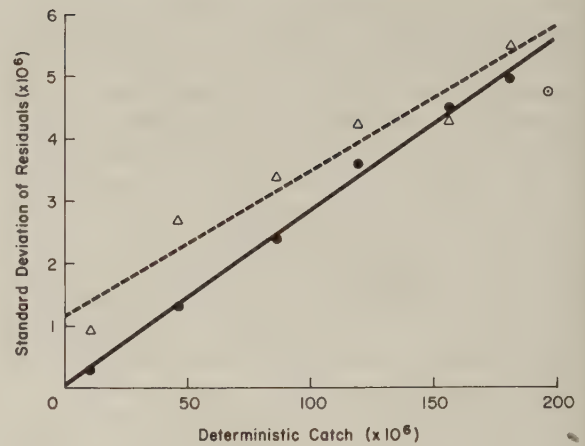


FIGURE 1.—Standard deviation of the residuals, ϵ_3 , plotted against the deterministic catch, $\hat{C}$, for statistical Model 3. ● = fishing effort below maximum sustainable yield (MSY) level. △ = fishing effort above MSY level. ○ = fishing effort at MSY level.

duces the maximum sustainable yield (MSY) ($C = 196 \times 10^6$). Regression analysis reveals that variance about regression, S_{yx}^2 , is highly significantly different between below and above MSY levels ($F = 9.90$; 4, 4 df; $\text{Pr} < 0.01$), but the regression coefficients, $\hat{b}$, are not significantly different ($t = 1.47$; 5 df; $\text{Pr} > 0.20$)—Table 2. The “above MSY” regression has a y -intercept, which must be zero, significantly different from zero ($t = 3.30$; 4 df; $\text{Pr} < 0.05$). It appears that Model 3, like Model 2, is valid up to $58,000 \leq f < 65,000$ (Figure 1).

TABLE 1.—Results of the stochastic catch simulation trials of the generalized production model.

Fishing effort f	Deterministic catch (10^6) $\hat{C}$	Deterministic population size (10^6) $\bar{P}$	Mean residual		Residual variance	
			$\bar{\epsilon}$ (10^6)	$\ln \epsilon$ (10^{-2})	$S^2(\epsilon)$ (10^{12})	$S^2(\ln \epsilon)$ (10^{-4})
1,000	9.6775	138.25	-0.0706	-0.7765	0.0844	9.1231
5,000	45.9375	131.25	-0.0599	-0.1713	1.7538	8.3187
10,000	85.7500	122.50	0.0143	-0.0218	5.7506	7.8882
15,000	119.4375	113.75	0.2155	0.1357	13.0288	9.0945
22,000	156.3100	101.50	-0.7088	-0.4956	20.4779	8.3884
29,000	181.1775	89.25	-1.1161	-0.6551	24.6701	7.5760
40,000	196.0000	70.00	-0.6563	-0.3646	22.6228	5.9845
51,000	181.1775	50.75	0.5173	0.2404	30.1457	9.1269
58,000	156.3100	38.50	-0.1051	-0.1044	18.4686	7.6021
65,000	119.4375	26.25	0.5438	0.3927	18.1894	12.5489
70,000	85.7500	17.50	0.2865	0.2577	11.5283	15.4564
75,000	45.9375	8.75	-0.3734	-0.9846	7.2114	34.2285
79,000	9.6775	1.75	-0.2770	-3.4017	0.8834	102.6439

TABLE 2.—Regression analysis for statistical Model 3 of standard deviation of catch residuals, $S(\epsilon_3)$, on deterministic catch, $\hat{C}$, with levels of fishing effort below and above that which produces the maximum sustainable yield (MSY).

Effort level regression	Degrees of freedom	Σx^2	Σxy	Σy^2	$\hat{b}$	Degrees of freedom	Sum of squares	$S^2_{y/x}$
Below MSY	5	21421.9	601.047	16.9475	0.028058	4	0.083566	0.020892
Above MSY	5	21421.9	498.146	12.4109	0.023254	4	0.826986	0.206746

In conclusion, the assumption of statistical Models 0 and 1 were rejected by the simulation study. Statistical Models 2 and 3 were found to be valid over a wide and similar range of fishing effort. Their range of validity includes up to and well beyond the level of fishing effort producing the MSY ($f = 40,000$), the most likely range in which a fishery would operate. Employing Model 3 has a theoretical advantage over Model 2 in a least-squares estimating procedure. With Model 3, the actual residual variance is minimized. Whereas with Model 2 the log-residual variance is minimized and the parameters are best least-squares estimates only in the transformed model. The theoretical advantage of Model 3 may serve as a criterion for choosing it when no other criteria exist.

Several additional simulation trials were made to demonstrate the relative degree of influence that random variability in each parameter exerts on the variance of the catch residuals. The upper two standard deviations of each stochastic variable was set equal to 25% of their mean, the level of fishing effort was set at 40,000 (MSY-producing level), and four trials of 500 time periods each were made. Each parameter in turn was allowed to vary with the remaining three constant (Table 3). The variation in catch was most sensitive to varying the exponent, m , and least sensitive to varying the catchability coefficient, q . This, of course, implies the relative precision of the parameters if they had been ac-

tual estimates. One should not, however, generalize on the order of precision since these results obtain specifically for the assumed probability distributions and expected values. This exercise does demonstrate a frequently employed method for implying which parameters, given their estimates, are most critical and perhaps deserving of additional independent estimation.

RESIDUALS EXAMINATION:
AN EXAMPLE

The data of catch, catch per unit effort, and fishing effort from the eastern tropical Pacific yellowfin tuna fishery (Pella and Tomlinson, 1969; Table 6) are plotted in Figure 2. Apparently the population and fishery dynamics are

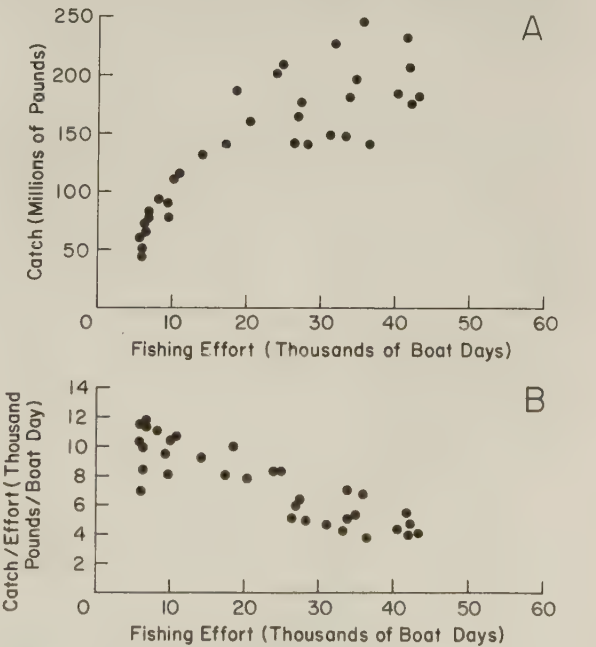


FIGURE 2.—Data from the eastern tropical Pacific yellowfin tuna fishery, 1934-67, plotted as (A) catch vs. fishing effort, and (B) catch per unit effort vs. fishing effort.

TABLE 3.—Catch residual variance produced by variation in each stochastic variable of the generalized production model.

Stochastic variable	Expected value	Approximate 95% range	Residual variance	
			$S^2(\epsilon)$ (10 ¹²)	$S^2(\ln \epsilon)$ (10 ⁻⁴)
μ	2.00	1.67-2.50	46.0834	11.9100
κ	5.60	4.20-7.00	36.8604	9.7958
π (10 ⁸)	1.40	1.12-1.75	30.7556	8.1498
γ (10 ⁻⁵)	7.00	5.60-8.75	17.1333	4.4781

well described by a production model—good relationships are observed in Figure 2. These data were used by Pella and Tomlinson in exemplifying their technique; for comparative purposes the same data are utilized here. The results of this section, however, should be considered as just an example and not a recommendation on management.

The parameters of the generalized production model for the tuna fishery were estimated by the Pella-Tomlinson computer program, GENPROD, replacing the fitting criterion, S , with those of each alternative statistical model [equations (11), (15), and (18)]. Each parameter was searched to five digits or until the improvement in S was less than 0.01% at three levels of numerical approximation in equation (5)— $N = 1, 3, 5$ —(Table 4). Increasing the precision of numerical approximation greatly changed the parameter estimates between $N = 1$ and 3, but only slightly between $N = 3$ and 5. The most sensitive parameter is H , followed in order by K , q , m , and r . Consequently, the estimates of the average environmentally limited maximum population size, $\bar{P}_\infty$, and average optimum population size $\bar{P}_{opt}$, vary with the level of pre-

cision. Pella and Tomlinson indicated that unreasonable estimates were obtained for the catchability coefficient, q , (presumably with $N = 1$) and made an arbitrary selection of a "reasonable" estimate. "Reasonable" catchability coefficients are obtained here with $N = 3$, making unnecessary the arbitrary selection of a reasonable estimate. The management implications of maximum equilibrium catch, C_{max} , and optimum fishing effort, f_{opt} , are surprisingly robust to the degree of precision of the numerical approximation. Schaefer (1957) mentioned previously, however, that these two management implications are robust to changes in the estimate of q in his estimating method; Pella and Tomlinson also mentioned the phenomenon for their technique. The S criteria values were reduced about 7% or less by choosing $N = 3$, as against $N = 1$ and reduced a negligible 0.2% or less by choosing $N = 5$ (S_1 and S_2 increased minutely due to the level of precision chosen for S). Obviously, the error due to approximation in equation (5), as previously stated, is negligible for these data with $N \geq 3$.

Turning to the effects of the alternative statistical models (with $N = 5$), it may be seen

TABLE 4.—Parameters and management implications of the generalized production model for the eastern tropical Pacific yellowfin tuna fishery, 1934-67, estimated with the Pella-Tomlinson technique (GENPROD) using four different statistical models and three levels of precision, N , in equation (5).

Model	Parameters					Management implications				$\bar{S}$ Criterion
	$\hat{m}$	$\hat{H}$ (10 ⁻⁵)	$\hat{K}$	$\hat{r}$	$\hat{q}$ (10 ⁻⁵)	$\bar{P}_\infty$ Pounds (10 ⁶)	$C_{\max}$ Pounds (10 ⁶)	f_{opt} Boat days	$\bar{P}_{\text{opt}}$ Pounds (10 ⁶)	
$N = 1$										
20	1.4	--	--	--	45.---	--	182.6	35,300	--	1.78... $\times 10^{16}$
0	1.4	2900.1	33.26	.820	27.00	44.6	182.6	35,200	19.2	1.7858 $\times 10^{16}$
1	1.9	0.17064	15.21	.904	21.69	52.7	186.2	33,210	25.8	4.7140 $\times 10^7$
2	1.6	34.502	16.65	.879	18.10	64.0	182.5	34,500	29.2	8.2214 $\times 10^{-1}$
3	2.0	0.00748	7.57	.865	11.50	101.2	191.5	32,900	50.6	7.8762 $\times 10^{-1}$
$N = 3$										
0	1.5	59.999	7.65	.842	7.36	162.7	184.5	34,660	72.3	1.7197 $\times 10^{16}$
1	2.2	0.00013	5.90	.921	10.00	113.0	188.6	32,200	58.6	4.5291 $\times 10^7$
2	1.8	0.17092	5.86	.952	7.70	147.4	184.0	33,800	70.7	8.0355 $\times 10^{-1}$
3	2.0	0.00408	5.59	.835	8.50	136.9	191.5	32,900	68.5	7.3769 $\times 10^{-1}$
$N = 5$										
0	1.5	55.802	7.28	.843	7.10	170.4	183.9	34,200	75.7	1.7185 $\times 10^{16}$
1	2.2	0.00010	5.38	.921	9.11	124.0	188.7	32,210	64.3	4.5296 $\times 10^7$
2	1.8	0.15820	5.61	.926	7.40	153.9	184.1	33,700	73.8	8.0371 $\times 10^{-1}$
3	2.1	0.00054	5.04	.845	8.10	142.7	192.6	32,700	72.7	7.3608 $\times 10^{-1}$

¹ $r = P_0/P_\infty$

² Pella and Tomlinson (1969; Table 5).

that the most sensitive estimate is H , followed in succession by the estimates of m , K , q , and r . The estimates of the management implications $C_{\max}$ and f_{opt} are, for all practical purposes, the same among statistical models, but less similar than among levels of precision. This may be offered as an argument against considering alternative statistical models. But consider the plot of the data in Figure 2; one could draw an average line by eye through the data and arrive at estimates of $C_{\max}$ and f_{opt} just as accurate as those estimated by the sophisticated least-squares search technique. The point is that with *good* data most rational statistical procedures should provide similar estimates of $C_{\max}$ and f_{opt} . One cannot be certain that this will be so with data of lesser quality or different range. The values of m which determine the shape of the yield curve, on the other hand, are very different between Models 0 and 3. This could have a significant effect on an economic analysis of the yield curve.

In the absence of other criteria for choosing a particular statistical model, the "fit"—least sum of squared residuals—is often selected (Glass, 1969), and is perhaps a reasonable criterion if the goal is interpolation. The goal here is to obtain the best possible parameter estimates in order to make, in essence, extrapolations or predictions. In the latter case the best criterion is not the "fit", but the degree of assumption fulfillment. Statistical Model 3 provided estimates that were least influenced by the addition of error—comparing the parameters' precision between N equalling 1 and 5—inferring the greatest confidence in its estimates. It was also seen from the simulation study that Model 3 best fulfilled the assumptions of a least-squares procedure. Model 3, ironically, "fits" the data the worst, although only by about 6%.

Pienaar and Thomson (1969) have suggested the utilization of an important tool for selecting a statistical model which best fulfills the assumptions of the estimating procedure—residuals examination. Various plots of the residuals suggested by Draper and Smith (1966) were made for the four statistical models (Figure 3). Each statistical model gives a mean residual near zero fulfilling one of the least-squares as-

sumptions (Figure 3A). Plots of residuals against time (Figure 3B) indicate: 1) variation increases with time in Model 0 from 1934 through 1961, violating the assumption of constant residual variance; 2) Model 1 tends to over-correct as there is a propensity for variation to decrease from 1940 through 1967; and 3) Models 2 and 3 are nearly identical in controlling time-oriented variation. Runs—consecutive residuals of the same sign—are evident in all four models, indicating violation of the assumption of residual independence. There are only ten runs in Model 3 giving a probability less than 0.01 that the arrangement of signs is random (Figure 3B). Draper and Smith (1966) suggest, however, that unless the ratio of degrees of freedom to number of observations is small (here 29/34), the effect can be ignored. The dependence of consecutive residuals is undoubtedly due to violation of the assumption of no time lags in the fish population. With changes in fishing effort the age structure of the population is altered as well. It might be possible to average out these effects by considering a time period longer than one year, say the average life-span of an individual. That would be about 3 years for a yellowfin tuna, the approximate mean length of the runs. However, that would also reduce the number of observations to eleven and the fishing effort, assumed constant in integration of the model, would vary considerably.

An increase in residual variation with deterministic catch is obvious for Model 0 (Figure 3C), again violating the assumption of constant residual variance. As in the time plot, Model 1 tends to over-correct for the phenomenon exhibited by Model 0. Models 2 and 3 stabilize the variance as might be expected. In the final plot, residuals against fishing effort, the same conclusions may be reached (Figure 3D).

Models 2 and 3 apparently fulfill the assumptions of the least-squares procedure while Models 0 and 1 violate the assumption of constant residual variance. Invoking the previously mentioned criterion for choosing between Models 2 and 3, the best statistical model for this fishery is Model 3.

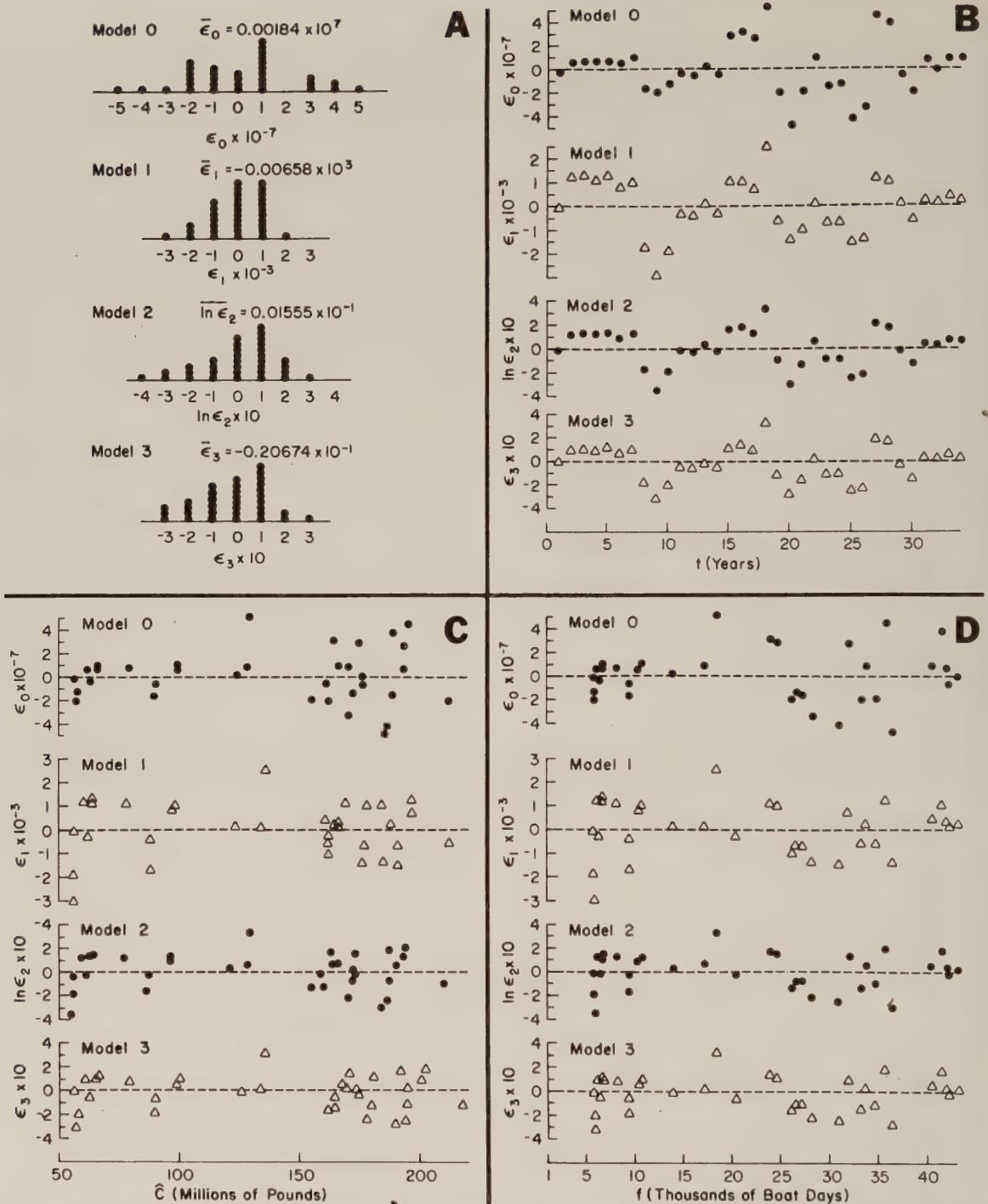


FIGURE 3.—Plots of residuals, ϵ_i , for statistical Models 0, 1, 2, 3, from the generalized production model for the eastern tropical Pacific yellowfin tuna fishery, 1934-67. A. Frequency distributions. B. Residuals against time. C. Residuals against deterministic catch, $\hat{C}_i$. D. Residuals against fishing effort, f_i .

The referee of this paper has raised an important point regarding application of the various statistical models to actual fishery data. In a non-overexploited fishery, generally, the quality and level of catch and effort values increase with time. Relatively speaking, Model 0 in this case places greater weight on more recent data than do Models 1, 2, or 3, and in the absence of any other criteria it might represent the intuitive choice. However, if the quality of the data were a more significant contributor to unequal residence variance than the statistical model, one would expect, in this case, a decrease in the residuals plotted for Model 0 against time, catch, and fishing effort in contrast to the apparent increase for the yellowfin tuna fishery (Figure 3). If one has reason to suspect a significant difference in quality of the data, as would be suggested by a decrease in the residual plots of Model 0, perhaps a solution is to partition the data at the point in time where a significant quality increase occurs. Then fit each set of data individually placing greater weight on the parameter estimates for the more recent set. The specter of the suitability of employing production models over long time periods is also raised by this point. But it is outside the scope of this paper and the reader is referred to the papers cited previously.

SUMMARY

In using a least-squares procedure for estimating parameters of a mathematical model, such as the Pella-Tomlinson technique, there are three assumptions about the residuals for obtaining the best least-squares estimates: 1) the residuals are independent, 2) the residuals have an expected value of zero, and 3) the variance of the residuals is constant (Anscombe and Tukey, 1963; Draper and Smith, 1966; Snedecor and Cochran, 1967). We have observed from the simulation study that two (of four alternative) simple statistical models which are biologically sound—Model 2 (using a logarithmic transformation) and Model 3 (weighting by the inverse of the squared deterministic catch)—fulfill the statistical assumptions for obtaining

good least-squares estimates of the generalized production model parameters over a wide range of fishing effort.

On applying these four statistical models in estimating the parameters of the generalized production model for the eastern tropical Pacific yellowfin tuna fishery, residuals examination revealed that the same two statistical models, Models 2 and 3, fulfilled the least-squares estimation assumptions. Models 0 (assumed by Pella and Tomlinson, 1969) and 1 did not. Model 3 was selected as the best model since it involves the direct minimization of the actual residual variance, and is therefore considered to be theoretically superior to Model 2.

Finally, anyone using the generalized production model and the Pella-Tomlinson estimating technique should be aware of, in addition to the proper statistical model, the effect of the value of N in equation (5) on the parameter estimates.

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Douglas G. Chapman, Director of the Center for Quantitative Science in Forestry, Fisheries and Wildlife, University of Washington, for the considerable consultation he gave during the course of this study and for his review of the final manuscript. Many others, especially the referee, who offered suggestions on clarification and improvement of the value of this study, are also extended my gratitude.

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ADDITIONAL DATA ON THE SPAWNING OF THE HAKE

JOHN S. MACGREGOR¹

ABSTRACT

In January 1970 samples of hake were taken off southern and central Baja California to study fecundity. In the southern area female hake as small as 130 mm standard length contained developing eggs, and all females longer than 140 mm contained such eggs. There is a marked cline in size at first maturity of hake along the Pacific Coast (hake in the Pacific Northwest exceed 400 mm before reaching maturity). Seventeen female hake 130 to 202 mm long taken in the southern area contained from 3,400 to 19,500 eggs or 229 per gram of fish; 11 females 222 to 305 mm from the central area contained from 3,500 to 110,000 eggs or 243 per gram of fish. In previously published data 22 female hake 346 to 688 mm from northern Baja California contained from 33,000 to 496,000 eggs or 192 eggs per gram of fish. There are no significant differences in fecundity among the three areas. The hake spawns once a year with over 98% of the spawning taking place between January and April.

In 1966, I published data on the fecundity of 22 female hake taken off northern Baja California. In 1970, additional samples of hake were obtained from central and southern Baja California to determine if there were geographic differences in hake fecundity in the offshore waters of Baja California.

The hake samples taken off southern and central Baja California in January contained prespawning females from which estimates of fecundity were obtained.

The hake from northern Baja California for which fecundity data have been published (MacGregor, 1966) appear to be identical to those taken off southern and northern California, while those taken farther to the south are different with respect to growth rate and size at first maturity and, in fact, have been described as a different species (Ginsberg, 1954).

The female hake for which fecundity determinations were made were taken by trawl from the research vessel *David Starr Jordan*. Station J-45-13 at lat 26°07' N, long 113°07' W was sampled January 11, 1970. Station J-45-27 at lat 28°44' N, long 115°15' W was sampled January 16, 1970. Methods for estimating fecundity of the samples were essentially the same as used previously (MacGregor, 1966).

Previous data on hake fecundity (MacGregor, 1966) were obtained from samples taken by the research vessel *John N. Cobb* (Berry and Perkins, 1966). Station C-58-23 at lat 31°49' N, long 117°53' W was sampled March 21, 1963. Station C-58-29 at lat 29°46' N, long 116°01' W was sampled March 23, 1963. Station C-58-31 at lat 29°35' N, long 116°00' W was sampled March 25, 1963.

FECUNDITY

The range for fecundity data for samples J-45-13 (Table 1) and J-45-27 (Table 2) compare with Cobb 1963 samples (MacGregor, 1966) as follows:

	J-45-13	J-45-27	Cobb 1963
Standard length (mm)	130 to 202	222 to 305	346 to 688
Weight (g)	22.1 to 57.0	88.0 to 221.0	300 to 2,750
Gonad weight (g)	0.928 to 4.002	3.279 to 22.710	13.1 to 196.8
Advanced eggs	3,419 to 19,564	3,496 to 110,017	33,000 to 496,000
Eggs per gram of fish	141 to 343	38 to 498	83 to 556

There is no overlap in the ranges of standard length and fish weight of the samples from the three localities. The number of advanced eggs in the ovaries tends to increase with size of fish both within and between samples. However, because of the great variation in the numbers of advanced eggs among the individual fish, there is considerable overlap in gonad weight and numbers of advanced eggs between successive samples.

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TABLE 1.—Fecundity data for 17 female hake taken at Station J-45-13 (lat 26°07' N, long 113°07' W) January 11, 1970.

Standard length	Weight	Gonad weight	Gonad index	Advanced eggs		Eggs per gram of fish
				Size range	Number	
mm	g	g		mm		
130	22.1	0.928	4.2	0.50-0.67	3,419	155
131	23.3	1.345	5.8	.57-.73	7,049	303
135	25.2	1.320	5.2	.57-.77	3,564	141
137	22.4	1.518	6.8	.50-.67	6,469	289
137	29.3	1.795	6.1	.53-.70	7,139	244
138	24.8	0.987	4.0	.53-.70	4,048	163
140	28.8	1.350	4.7	.53-.70	4,956	172
140	34.5	2.241	6.6	.57-.77	7,926	230
141	30.5	1.222	4.0	.50-.70	4,534	149
150	21.2	1.198	5.7	.53-.73	4,257	201
150	37.1	2.481	6.7	.53-.73	10,864	293
160	31.5	1.875	6.0	.53-.73	6,447	205
162	30.3	1.957	6.5	.57-.73	7,633	252
170	32.3	2.000	6.2	.50-.70	8,741	271
172	37.7	1.863	4.9	.47-.70	10,731	285
174	37.7	2.000	5.3	.50-.70	7,559	201
202	57.0	4.002	7.0	.50-.67	19,564	343

TABLE 2.—Fecundity data for 11 female hake taken at Station J-45-27 (lat 28°44' N, long 115°15' W) January 16, 1970.

Standard length	Weight	Gonad weight	Gonad index	Advanced eggs		Eggs per gram of fish
				Size range	Number	
mm	g	g		mm		
222	100	8.663	9.7	0.50-0.67	29,399	294
230	88	7.011	8.0	.50-.73	23,253	264
233	104	8.488	8.2	.60-.77	29,450	347
238	91	3.279	3.6	.57-.73	3,496	38
245	105	6.292	6.0	.50-.70	17,511	168
247	120	5.956	5.0	.60-.80	18,264	152
252	110	9.341	8.5	.53-.77	43,014	391
268	147	5.308	3.6	.60-.80	13,747	94
275	146	11.860	8.2	.57-.83	38,959	286
287	176	12.972	7.4	.67-.83	24,877	141
305	221	22.710	10.3	.57-.80	110,017	498

The mean number of advanced eggs per gram of fish is 229 for sample J-45-13, 243 for sample J-45-27, and 192 for the Cobb 1963 samples. Owing to the great range of individual values in each of the samples, there is no significance in the differences between the means. The mean for the 50 fish in the three samples is 216 eggs per gram of fish. The standard error of the mean is 15 eggs or about 7%. In spite of some rather low fecundities, the distribution of eggs per gram of fish approximates a normal distribution indicating that these low counts are within the limits of expected variation.

Eggs/gram	Percent frequency
< 50	2
100	24
200	38
300	30
400	2
500	2
>550	2

The low counts apparently did not result from partial spawning of the advanced mode because they were found in fish that were not yet ripe.

RATIOS

To obtain estimates of the numbers of advanced eggs in the hake ovary, first about 100 yolked eggs in the sample were measured in order to delimit the distributions of advanced- and small-yolked eggs. Then the additional advanced-yolked eggs in the weighed sample were counted. An estimate of the numbers of small eggs was obtained from the ratio of large to small eggs in the measured frequency distribution, but because this estimate is based on relatively few eggs it is less accurate than the estimates of advanced eggs. Estimates of the numbers of small eggs per gram of fish ranged from

33 to 766 and averaged 248. This compares with 38 to 556 with an average of 216 for the advanced eggs for all samples.

The correlation obtained by MacGregor (1966) between percentage of eggs in the advanced mode and advanced eggs per gram of fish has no mathematical significance because both variables are related. Actually there is no relation between the number of advanced eggs and the number of small eggs. Ovaries containing either high or low numbers of advanced eggs per gram of fish may contain either high or low numbers of small-yolked eggs. However, the conclusion that the great variation in the ratios of large to small eggs makes multiple spawning unlikely seems to be valid.

Of 366,093 hake larvae taken on monthly cruises in the 6 years 1951 through 1956, 87% were taken in January, February, and March, 11.5% in April, and the remaining 1.5% in the remaining 8 months. Several large samples of hake taken off California in April 1970 showed that the advanced eggs were no longer present in the ovaries of the females while the smaller yolked eggs were. Because there is no evidence of further egg development in the ovaries and no evidence of heavy spawning subsequent to April in the plankton, we must assume that these small-yolked eggs are resorbed following spawning of the advanced mode.

SIZE AT FIRST MATURITY

One hundred thirty-six hake from sample J-45-13 (southern Baja California) were examined for stage of sexual maturity. Eighty hake from sample J-45-27 (central Baja California) and an additional seven hake taken in 1961 near Cedros Island (central Baja California) were also examined (Table 3).

In sample J-45-13 the largest immature male was 127 mm in length, and the largest immature female 138 mm. The smallest maturing male was 119 mm, and the smallest maturing female 125 mm. All males 129 mm and longer and all females 140 mm and longer were maturing.

In sample J-45-27 the range of fish length was not as good for determining size at first maturity. The smallest maturing male was 137 mm long, and it appeared that all males 159 mm and longer were mature. The smallest mature female was 222 mm in length, and all females of this length and longer were maturing. However, on the basis of the 1961 sample it appears that all females were maturing at some length between 202 and 222 mm.

It is difficult to determine the size at first maturity for hake off northern Baja California and California because very few fish of suitable size, taken during the spawning season, were available. For off-season hake the gonad index may

TABLE 3.—Size at first maturity. Southern Baja California, sample J-45-13, lat 27°07' N, long 113°07' W, January 11, 1970. Central Baja California (Cedros Island area), sample J-45-27, lat 28°44' N, long 115°15' W, January 16, 1970, and sample B-6111-3, Cedros Island, November 25, 1961. Males judged mature or immature by size and appearance of testes. Females judged immature if largest eggs in ovary were 0.20 mm or less in diameter (not yolked); probably maturing if maximum egg diameter was 0.23 to 0.47 mm; mature if maximum egg diameter was 0.50 mm or larger. Immature, not sexed: gonads not developed enough so that sex can be determined by gross examination.

Sample	Males			Females			Not sexed		
	Standard length	Immature	Mature	Standard length	Maximum egg diameter (mm)			Standard length	Immature
					0-0.10	0.30-0.47	0.50-0.77		
J-45-13	mm			mm				mm	
	119-127	6	6	122-124	6	0	0	100-118	19
				125-128	8	1	0		
	129-166	0	50	130-138	9	4	6		
				140-202	0	0	21		
					Maximum egg diameter (mm)				
					0-0.20	0.37	0.67-0.83		
J-45-27	137-140	1	1	172-197	3	0	0	120-138	42
	159-307	0	21	222-228	0	1	1		
				230-305	0	0	10		
B-6111-3				236	0	0	1	148-202	6

indicate maturity. Generally, hake with gonad indices of less than 0.5 (i.e., the gonad weight is less than 0.5% of the fish weight) do not contain yolked eggs in their ovaries. There is no yolk in eggs up to 0.20 mm diameter, and fish containing such eggs a few months before or after the spawning season may be considered immature. Eggs in an ovary having a gonad index of 0.8 have a maximum diameter of about 0.34 mm and at a gonad index of 1.5, about 0.43 mm maximum diameter. The eggs generally are not large enough to count (over 0.65 mm diameter for the largest eggs in the ovary) until the gonad index is about 3.5, and then only for fish having a fecundity of less than 100 eggs per gram of fish. If we apply the same criterion (a gonad index of less than 0.5 as indicating an immature fish) to the males, we can roughly estimate the size at maturity for off-season hake.

Applying this criterion of maturity to a number of miscellaneous hake samples taken in the off-season for spawning off northern Baja California and southern California, it appears that all males 285 mm and longer and all females 340 mm and longer were mature.

Best (1963) estimated that all hake, both males and females, taken off northern California were mature at 400 mm total length (about 360 mm standard length). The length at which all fish are mature could be somewhat less as he had a limited number of smaller fish in his samples.

Nelson and Larkins (1970) found that all fish of 450 mm total length (about 405 mm standard length) were mature in the Pacific Northwest. Apparently they also had few smaller fish to work with, and the length at which all fish are mature could be somewhat less.

DISCUSSION

The mean number of eggs per gram of fish was not significantly different among the three samples, J-45-13, J-45-27, and Cobb 1963. These three samples were taken in widely separated localities, and although they were similar with respect to relative fecundity, questions have been raised as to the distinctness of the north Pacific hake with respect to race or even species.

Ginsberg (1954) assigned the north Pacific hake to two species based on morphometric and meristic characters. His descriptions were based on 12 specimens of *Merluccius productus* taken off Washington, Oregon, and California, as far south as San Diego, and eight specimens of *M. angustimanus* taken in the Gulf of Panama, the Gulf of California, off the Pacific Coast of Baja California, and off Del Mar, Calif.

Ahlstrom and Counts (1955) could find no evidence of more than one species of hake in their extensive collections of eggs and larvae taken between San Francisco and the southern tip of Baja California. All of the small fish that had fully developed dorsal and anal fins had fin ray counts that fell within the range of *M. productus* but outside of the range of *M. angustimanus* as given by Ginsberg.

F. H. Berry (unpublished data) studied numerous additional specimens of hake from Baja California and California. He concluded that *M. productus* and *M. angustimanus* were the same species and the differences in meristic and morphometric characters, used by Ginsberg to separate the species represented a latitudinal cline.

There was certainly a marked cline in size at maturity for the hake used in this study, especially when these data are compared with data given by other authors. The size at which all fish were mature was as follows:

	Sex	Standard length
Southern Baja California	males	129 mm
	females	140 mm
Central Baja California	males	159 mm
	females	202-222 mm
Northern Baja California and southern California	males	285 mm
	females	340 mm
Northern California (Best, 1963)	both	360 mm
Pacific Northwest (Nelson and Larkins, 1970)	both	405 mm

The differences between areas are so great that the roughness of some of the estimates does not affect the conclusion that these differences are very real.

The distribution and growth patterns of the European hake, *M. merluccius*, is similar to that of *M. productus* in many ways. The European hake ranges from Norway to at least Mauritania in Africa (Hart, 1948) while *M. productus*

ranges from Alaska to at least the southern tip of Baja California. Both species grow to much larger sizes in the northern parts of their ranges, but are much smaller in their southern ranges. *M. merluccius* in the Mediterranean Sea is smaller than the north Atlantic form, and both the southern Baja California and African coasts apparently produce dwarf races of their respective hake species.

Most recent information from the Guinean Trawling Survey (Williams, 1968) shows that there are continuous populations of hake from Norway to South Africa. However, the hake taken off the west coast of Africa are ascribed to several species other than *M. merluccius*. The West African hake, *M. polli* and unidentified *M.* spp. were taken throughout the survey area from the Gambia border to the Congo. The Senegal hake *M. senegalensis* was taken in the northern areas between the Gambia border and southern Liberia with one questionable record from Nigeria, and the South African hake *M. capensis* was taken in the southern areas between Cameroun and the Congo. A sample of 50 *M. senegalensis* averaged 26.3 cm total length (range 18 to 27), and a sample of *M. polli* averaged 41.7 cm total length (range 35 to 49).

SUMMARY

The north Pacific hake, *Merluccius productus*, ranges from Alaska to at least southern Baja California.

The fecundity of individual hake varied greatly off Baja California and southern California, but there was no significant difference in average fecundity among the samples taken from widely separated sampling stations in this area. Estimates of the number of advanced

eggs contained in 50 prespawning hake averaged 216 eggs per gram of fish.

Average size at first maturity for female hake varied from 133 mm standard length off southern Baja California to about 340 mm off northern Baja California and southern California. Males appeared to mature at smaller sizes 128 mm in the south to 285 mm in the north.

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THE LOW-TEMPERATURE THRESHOLD FOR PINK SALMON EGGS IN RELATION TO A PROPOSED HYDROELECTRIC INSTALLATION

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ABSTRACT

A proposed hydroelectric installation in southeastern Alaska would alter the seasonal pattern of stream temperatures and pose a threat to the natural production of pink salmon, *Oncorhynchus gorbuscha*. Analysis of experiments reported in the literature indicated that such an installation might lower stream temperatures below the threshold normal for the embryonic development of pink salmon. Our experiments with pink salmon eggs incubated in refrigerated water showed that the eggs required initial temperatures above 4.5° C for normal embryonic development. An increase in mortalities and in alevins with spinal deformities occurred when initial incubation temperatures were 4.5° C and lower; initial incubation at 2° C resulted in complete mortality. The proposed hydroelectric installation could result in temperatures as low as 4.5° C during spawning and initial incubation and could therefore be expected to cause an increase in mortality and the occurrence of deformed alevins. The low temperature would be followed by higher than normal winter incubation temperatures, which would have an unknown effect on the time of emergence of fry. A tunnel intake designed to draw water of a desirable temperature on demand would be required to protect salmon.

In 1964 the Bureau of Reclamation (now the Alaska Power Administration) started feasibility studies on a hydroelectric installation on Lake Grace, 51 km northeast of Ketchikan, Alaska. Grace Creek, the lake's outlet stream, enters the sea 4 km from Lake Grace. An impassable falls 2 km above tide water prevents migrating fish from reaching Lake Grace. Below the falls, the creek provides important spawning, incubation, and rearing areas for salmonids, especially pink salmon, *Oncorhynchus gorbuscha*.

The proposed dam would divert practically all of the water from Grace Creek through a hydroelectric plant and back into Grace Creek about 1.2 km from tide water. Because of the design of the dam and of the water intake, the temperature of the lower 1.2 km of the creek could be changed to lower than normal in summer and fall and higher than normal in winter.

Analysis of the studies by Combs and Burrows (1957) and Combs (1965) on the relation between temperature and the development of salmonid embryos indicated that when the hy-

droelectric facility is constructed, water temperatures in the principal spawning areas of Grace Creek might be too low for normal embryonic development. We therefore estimated the temperatures likely to occur in Grace Creek. Because these temperatures seemed critically low, we conducted laboratory experiments to determine precisely the low-temperature threshold or minimum temperature for the normal development of embryos of pink salmon, the major species in Grace Creek.

In this report we analyze the effects of the proposed installation on the temperature regime of Lake Grace and Grace Creek and describe the threshold temperatures for development of pink salmon embryos, and then relate the two studies and discuss their implications.

EFFECTS OF PROPOSED INSTALLATION ON TEMPERATURE REGIME OF LAKE GRACE AND GRACE CREEK

To consider the effects of the installation on the temperature regime, we compared the seasonal temperature pattern of Grace Creek under normal conditions with the temperatures likely

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to occur when the proposed power plant is in operation.

The daily maximum and minimum temperatures for Grace Creek (U.S. Geological Survey, 1966: 15; 1967: 17; 1968: 16) for the first and second half of each month between April 16, 1965, and March 31, 1967, were averaged to determine the annual temperature pattern under normal conditions (Figure 1). The highest temperature for this period was 17.8° C and the lowest was 0.6° C. Normally, Grace Creek temperatures reach a maximum of about 15° C at the start of the spawning season in mid-August, and decline to 10° C at the end of the spawning season the first week of October. After the spawning season, stream temperatures continue to fall, reaching about 6° C by late November and about 1.5° C in midwinter, when ice cover forms on Lake Grace during most years. The average stream temperature is about 1.5° C from the end of December until the end of March.

If the proposed power development on Lake Grace is completed, it would significantly change the physical dimensions and waterflow of the lake. The dam at the outlet of Lake Grace would raise the lake surface from the present elevation of 131.1 m (mean sea level) to a maximum elevation of 152.4 m and increase its surface area from 668 to 1,046 ha. Water would be diverted through a pressure tunnel and exposed penstock to a powerhouse on Grace Creek, 1.21

km downstream from the dam. The intake elevation of the diversion tunnel would be constant at 125.0 m, but the elevation of the reservoir surface would change from 131.4 to 152.4 m as the active reservoir capacity of $1.84 \times 10^8 \text{ m}^3$ is used. The maximum depth of the natural lake is 129.5 m.

Most of the water in the stream below the powerhouse would come through the powerhouse and would therefore be similar in temperature to the water at the tunnel intake level of the lake. To predict the temperature of this water, we obtained temperature-depth profiles from Lake Grace during the freshwater phase of the reproductive cycle of pink salmon. Profiles were taken on July 27, 1961, August 12, 1965, September 16, 1965, October 8, 1965, November 18, 1965, and March 25, 1965 (Figure 2). In addition to the temperature-depth profiles, we obtained thermograph records which indicated surface water temperatures attained 4° C November 28, 1965, and again May 28, 1966. These thermograph records provide our best estimate of the dates of autumn and spring overturn of Lake Grace. Although these data were not all taken during a single reproductive cycle of pink salmon, we feel they are representative. These predicted estimated temperatures of the lake waters that would enter the intake are probably higher than would actually occur because increasing the depth of the lake increases its thermal capacity and results in colder deep water (Hutchinson, 1957). No correction was made for the cooling effect of deeper water.

The actual surface elevation of the reservoir and therefore the depth of the tunnel intake would depend on operational requirements of the power plant and the flow of water into Lake Grace. The fixed tunnel intake at the 125.0-m elevation could be under 6.4 to 27.4 m of water because the proposed active reservoir elevation varies from 131.4 to 152.4 m. We allowed for these fluctuations in using the temperature-depth profiles as an indication of water temperatures at tunnel intake depth.

The project development plan for Lake Grace included a graphic model of simulated reservoir water surface elevations for each month from

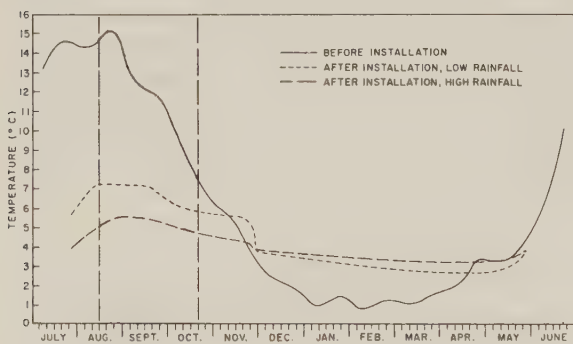


FIGURE 1.—Average annual temperature pattern of Grace Creek, based on temperature records from April 16, 1965, through March 31, 1967, and temperature patterns that might prevail during years of extreme high and low rainfall if water is drawn from a reservoir at the 125.0-m elevation.

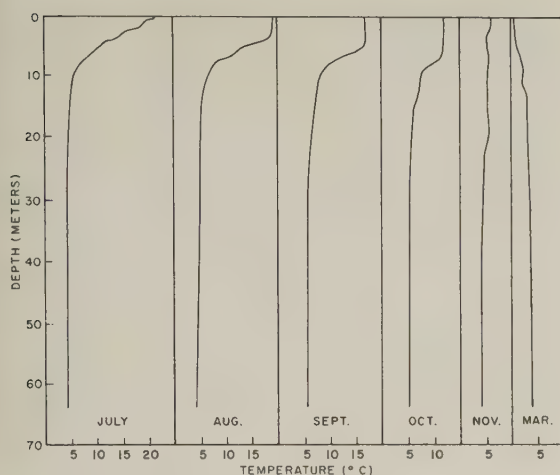


FIGURE 2.—Temperature-depth profiles from Lake Grace during months of the freshwater reproductive cycle of pink salmon.

January 1928 through December 1964.³ The basis of the model included records of streamflow and climate from Grace Creek and several nearby streams and also a computer study of the monthly operation of the power plant. We have chosen the year of lowest water level (1948) and the year of highest water level (1934) from the surface elevation model to illustrate the range of temperature patterns that might be caused by fluctuating water levels (Figure 1). The simulated water levels and depth of water above the entrance to the intake tunnel at 125.0 m used to predict water temperatures are shown in Table 1.

In Grace Creek, the probable result of the power plant would be lower than normal temperatures during summer and fall when salmon eggs are beginning their development and higher than normal temperatures during the winter when eggs and alevins are completing their development. Temperatures of Grace Creek during the normal spawning season currently range from 15° to 10° C, but under the conditions of power plant operation, temperatures would be from 7° to 5° C. During the first month after spawning, temperatures normally range from

10° to 6° C, but under the altered conditions they may be reduced to only 6° to 4.5° C. The predicted temperatures for the winter incubation period, 3° to 4° C, would be consistently higher than the 1° to 3° C in the unaltered stream.

LOW-TEMPERATURE THRESHOLD FOR NORMAL DEVELOPMENT OF EMBRYOS

Several workers have studied the low-temperature threshold for normal development of embryos of fishes. In this section we review their findings and describe our laboratory experiments with pink salmon.

EXPERIMENTS BY OTHER WORKERS

An important aspect of the effects of low temperatures is the stage of development at the time

TABLE 1.—Estimated water temperatures that would have prevailed at the 125.0-m elevation (mean sea level) of the proposed tunnel intake of Lake Grace if the proposed power plant had been in operation during the year of lowest water level (1948) and the year of highest water level (1934) from 1928 to 1964.

Month	Water surface elevation (m)		Depth of water above tunnel intake (m)		Water temperature at tunnel intake (° C)	
	1948	1934	1948	1934	1948	1934
July	135.6	152.4	10.4	27.4	5.6	3.9
August	134.1	152.4	9.1	27.4	7.2	5.0
September	137.2	150.9	12.2	25.9	7.2	5.6
October	141.7	152.4	16.8	27.4	6.1	5.0
November	143.2	152.4	18.3	27.4	5.6	4.4
March	135.6	149.4	10.7	24.4	2.8	3.3

the critical temperature is imposed on the embryo. Combs and Burrows (1957) associated high mortalities and gross anomalies in embryos of chinook salmon with low temperatures during the pregastrula stages; they used a significant rise in mortality in defining the low-temperature threshold. Combs (1965), Efimov (1962), and Price (1940) found that salmonid eggs were most sensitive to low temperature in the blastula and early gastrula stages. These authors demonstrated that once gastrulation is well underway, the embryos can tolerate temperatures close to freezing.

³ Alaska Power Administration. 1968. Lake Grace Project, Alaska. On file, Alaska Power Administration, Federal Building, Juneau, Alaska 99801.

Adverse effects of low temperatures during certain stages of development were also observed for other fishes. Kinne and Kinne (1962) exposed embryos of the cyprinodont *Cyprinodon macularis* to different temperature-salinity-oxygen combinations and found a period of "low thermal stability," which we presume to mean low resistance, in embryos exposed to critically low temperatures during early development (fertilization to gastrulation). Stockard (1921) conducted a number of experiments with eggs of the cyprinodont *Fundulus heteroclitus* which he placed in a refrigerator at 5°, 7°, and 9° C for various lengths of time and at various stages of development. Development was almost, if not completely, stopped at 5° C and greatly slowed at 9° C. Exposure to 5° C just after gastrulation commenced was not noticeably injurious, but exposure to low temperatures during earlier stages resulted in increased mortalities and gross anomalies among survivors.

Piavis (1961) incubated sea lamprey eggs at various constant temperatures and learned that viable burrowing larvae could be produced at 15.6° C but not at 12.8° C. McCauley (1963) also explored the lethal temperature limits of embryonic sea lamprey. He found that the narrow range of constant temperature, 15.0° to 25.0° C, necessary for successful hatching may be extended to 12.2° to 25.6° C if gastrulation is completed before the eggs encounter temperature extremes.

The work of Tåning (1952) on the effects of temperature on the development of *Salmo trutta trutta* supports Stockard's conclusion that the earlier the stage of development is arrested, the more severe will be the effect.

The lowering of temperatures in Grace Creek by the proposed hydroelectric plant would be greatest just before and during gastrulation of the pink salmon embryos (Figure 1).

EXPERIMENTS IN LABORATORY ON PINK SALMON

We conducted an experiment in the laboratory to determine if pink salmon eggs could survive and develop normally under the projected thermal regime for Grace Creek. The eggs for the

study came from two pink salmon collected September 7, 1966, from Grace Creek. The eggs were thoroughly mixed and fertilized by sperm from two males in the field.

Embryonic development had begun before the eggs were placed in the experimental array because the temperature in the transporting container ranged from 7° to 12° C (average, 10.8° C) during the 10-hr trip to the laboratory. According to Soin (1954) the first cleavage division occurs in pink salmon eggs about 7 hr after fertilization at 11° C. Knight (1963) showed that about 2.5 hr elapse between successive cleavage divisions in rainbow trout eggs at 12.2° C. Therefore, we estimate, but did not confirm, that the Grace Creek pink salmon eggs completed two cleavage divisions before they were transferred to the controlled temperatures of the experiment.

The eggs were incubated in 55- and 42.5-mm diameter Buchner funnels with perforated plates. Each of the large funnels was stocked with 120 eggs and each of the small ones with 25 eggs. The water was introduced through the stem of the funnel to produce an upwelling flow through the plates that supported the eggs. The water was not recirculated, and flow rates⁴ were set to deliver an apparent velocity of about 200 cm/hr to the eggs. Dissolved oxygen content of the water as it entered the funnels was above 8 ppm at all times.

The experiment involved 16 treatments consisting of four initial incubation temperatures each with four exposure periods. The four temperatures were ambient,⁵ 4.5°, 3.0°, and 2.0° C; and the four exposure periods were 15, 27, 37, and 103 days. All of the exposure periods began 10 hr after fertilization on September 7, 1966. When the experimental cold treatment for each lot was completed, the eggs were transferred to ambient temperature to complete their incubation. Temperatures were recorded continuously; the daily means (Figure 3) were usually within $\pm 0.5^\circ$ C of the planned levels. In-

⁴ Apparent velocity was obtained by dividing the rate of flow to the egg container in cubic centimeters per hour by the cross-sectional area of the container in square centimeters.

⁵ Ambient temperature is the unmodified temperature of the laboratory water supply. See Figure 3.

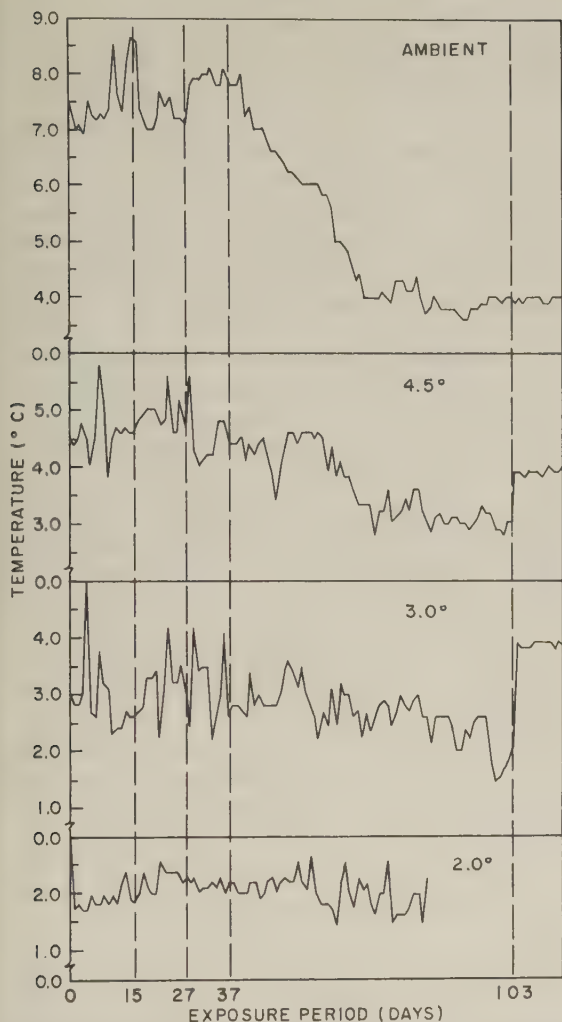


FIGURE 3.—Four initial incubation temperatures to which pink salmon eggs were subjected for four exposure periods in laboratory experiments on effects of low temperature on growth and development. Dashed lines indicate when individual lots were transferred to ambient temperature (see footnote 5).

frequent variations as great as $\pm 2^{\circ}\text{C}$ for 1 day were observed. Mean temperatures for the 37-day period September 7 through October 14 were within $\pm 0.2^{\circ}\text{C}$ of the planned levels. Ambient temperature, initially 6.9° to 8.7°C , dropped gradually to the usual winter level of 4.0°C by mid-November.

We made no direct observations of the stages of development of the embryos when they were

transferred to ambient temperature (on days 15, 27, 37, and 103), but in a separate experiment we observed development of pink salmon embryos in relation to temperature. At temperatures approximating ambient in the present experiment, the eggs began gastrulation about the 10th day and completed gastrulation about the 26th day. Eggs incubated initially at 4.5°C began gastrulation about the 21st day and completed gastrulation about the 45th day. Eggs incubated initially at 3.0°C began gastrulation about the 34th day and completed gastrulation about the 62d day.

We controlled water temperatures during incubation by mixing chilled and unchilled water in the intake line to each incubation funnel. A continuous flow of fresh water chilled to $1.0^{\circ} \pm 0.3^{\circ}\text{C}$ was obtained by operation of a 1/3-hp refrigeration unit. The cooling coils and agitator propeller were suspended in an insulated 20-gal fiberglass tank. Unchilled or ambient water was introduced through Y fittings to produce the required temperature for experimental lots of eggs.

Eggs incubated entirely at ambient temperature were first to hatch—the midpoint of hatching occurred December 9, 1966, 94 days after fertilization. The last eggs to hatch were from the group that was incubated initially at 3.0°C for 103 days. The survivors of the prolonged cold treatment hatched February 8, 1967, 154 days after fertilization.

Mortalities were inversely related to initial incubation temperatures. None of the eggs incubated at 2.0°C survived (Table 2); at 3.0°C , about 75% died; and at 4.5°C , about 10% died. Average mortality of eggs incubated entirely at ambient temperature was only 3%.

The occurrence of developmental anomalies was also associated with severity of the initial cold treatments. No alevins were produced in the 2°C treatment lots. Mild spinal deformities, various degrees of spinal curvature in the vertical plane, occurred in the 3.0°C and 4.5°C lots. The spinal flexures were not always severe enough to be easily recognized as deformities, but the deformity caused the lengths of alevins in these lots to be less uniform than the lengths of alevins in the ambient temperature lots.

TABLE 2.—Percentage mortality from fertilization to hatching of pink salmon in relation to initial incubation at low temperatures (number of eggs in each lot in parentheses).

Exposure period (days)	Percent mortality at temperature treatment ¹			
	Ambient	4.5° C	3.0° C	2.0° C
15	4 (24)	12 (24)	71 (24)	100 (24)
27	0 (25)	12 (25)	77 (22)	100 (25)
37	5 (20)	10 (21)	60 (20)	100 (21)
103	2 (41)	7 (41)	82 (40)	100 (40)

¹ See Figure 3 for temperature regime for each treatment.

Range in lengths among alevins in the ambient temperature lots was 20.9 to 22.9 mm, but range in length in the 3.0° C lots was 15.1 to 22.3 mm (Table 3).

Because of the increased mortality and abnormal embryonic development of Grace Creek pink salmon eggs at temperatures of 4.5° C and

TABLE 3.—Ranges in lengths (millimeters) of alevins from eggs treated at four temperatures (number of alevins measured in parentheses).

Exposure period (days)	Ranges in total length (mm) at—			
	Ambient temperature	4.5° C	3.0° C	2.0° C
15	21.3-22.6 (10)	20.8-23.2 (10)	18.4-21.9 (7)	—
27	21.1-22.8 (10)	21.0-23.1 (10)	20.5-21.7 (5)	—
37	20.9-22.3 (10)	19.3-22.3 (10)	19.4-22.3 (8)	—
103	21.0-22.9 (10)	20.2-22.4 (10)	15.1-20.3 (7)	—

lower, we conclude that initial incubation temperature for these eggs should be higher than 4.5° C. This is in agreement with the 4.4° to 5.9° C threshold for normal development of sockeye and chinook salmon eggs found by Combs (1965).

DISCUSSION

The proposed Grace Creek hydroelectric power plant focuses attention on a fishery problem that may become increasingly important if more hydroelectric plants are to be built on Alaska

streams. Where water for power generation is drawn only from the deeper and colder waters of reservoirs, the resulting stream temperatures would be lower than normal during the salmon spawning season and initial incubation period (Figure 1).

At Grace Creek the expected changes in water temperature could affect salmon in several ways. Delay in ripening of gonads of the adults after they enter the streams because of the lowered temperatures (Reingold, 1968) might result in late spawning. The low initial incubation temperatures would further delay development of embryos, but if normal cleavage were not disrupted this delay could be offset by the higher winter temperatures. The net effect on time of emergence and seaward migration of the fry is not known. The predicted initial incubation temperature of 4.5° to 7.2° C (Figure 1) for pink salmon eggs at Grace Creek includes the temperature 4.5° C, at which we detected abnormal development and increased mortality of the embryos. Measures should therefore be adopted to prevent deleterious temperature changes. Provision of an intake designed to draw water of a desirable temperature on demand is suggested as minimum action to protect the salmon.

ACKNOWLEDGMENTS

Theodore R. Merrell, Jr., generously gave of his time and thoughts in discussions of the problem. Charles J. DiCostanzo critically reviewed the manuscript and made suggestions to improve clarity of presentation.

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PRIMARY PRODUCTION IN THE MID-SUBARCTIC PACIFIC REGION, 1966-68

JERRY D. LARRANCE¹

ABSTRACT

Primary productivity, chlorophyll *a*, net zooplankton, nutrients, and associated physical variables were measured on seven cruises in the mid-Subarctic Pacific Region in 1966-68. Most of the data were collected between lat 46° N and the central Aleutian Islands, although several measurements were made as far south as lat 40° N. Primary productivity and chlorophyll were higher in Aleutian coastal waters than in areas to the south, but no other major differences among upper zone domains were consistent seasonally. Production was low in winter, high in spring, and intermediate throughout the summer. Annual productivity was between 80 and 100 g C/m². Chlorophyll *a* concentrations changed only slightly except in March when chlorophyll was high during the early part of the phytoplankton bloom.

Low light intensities limited primary production during the winter, and zooplankton grazing appeared to limit production in summer and part of spring. Nutrients and light were always sufficient to support high productivity during spring and summer except in late summer when some nutrients, particularly nitrate, were very low south of lat 44° N; however, the productivity did not appear severely limited. The main source of phosphate replenishment in the upper layers during spring and summer was probably in situ regeneration by zooplankton rather than upwelled deep water.

The pelagic biota of the Subarctic Pacific Region has long been recognized as distinct from that in the Subtropical Region, and the Subarctic is thought to be generally more productive. Until the introduction of the carbon-14 technique by Steemann Nielsen (1952), however, no adequate means existed for directly measuring primary productivity in the open ocean. Since that time thousands of measurements of primary production have been made throughout the Tropical and Subtropical North Pacific. Measurements in the Subarctic Pacific have been fewer and more localized.

Koblents-Mishke (1965), who summarized data from the Pacific Ocean, estimated that primary productivity in the mid-Subarctic Region averaged about 150 to 250 mg C/m² per day or 55 to 91 g C/m² per year. She estimated average production in the Gulf of Alaska and along the Washington and Oregon coasts to be between 250 and 650 mg C/m² per day (90-240 g C/m² per year) and in the transition area of the southern Subarctic to be 100 to 150 mg C/m²

per day (35-55 g C/m² per year). As discussed by Koblents-Mishke, these estimates are rather imprecise because productivity at most of the stations was measured only at the surface and not throughout the euphotic zone and because many of the measurements were made in artificial light of various intensities. Comprehensive analyses of annual cycles were seldom possible because surveys have been made during all seasons in only a few studies.

From detailed year-round surveys, Anderson (in press) estimated annual primary production in oceanic waters off Washington and Oregon to be 125 g C/m². Mean annual primary production at Ocean Station "P" (lat 50° N, long 145° W) in the Gulf of Alaska was 48 g C/m² in 1960-66 (McAllister, 1969). Although primary productivity has been measured on several individual cruises through the region (McGary and Graham, 1960; Faculty of Fisheries, Hokkaido University, 1961; Doty, 1964; Koblents-Mishke, 1965), no previous time-series studies of productivity have been made in the central Subarctic Region west of Station "P."

Primary productivity, zooplankton abundance, and related physical and chemical oceanographic

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variables were measured on several cruises in 1966-68 within the Subarctic Region in conjunction with studies of abundance and distribution of Pacific salmon (genus *Oncorhynchus*). Productivity data are listed in Larrance (1971), zooplankton is discussed by Day (1970),² and physical data are listed in Ingraham and Fisk (1970). The objectives were to obtain an estimate of annual productivity and to detect what differences in levels of productivity, if any, occurred among several oceanographic areas identifiable by physical characteristics.

METHODS

Primary productivity and related variables were measured on cruises of the RV *George B. Kelez* in March, June, and September 1966;

² Day, D. S. 1970. Distribution of zooplankton from the mid-Subarctic Region of the Pacific Ocean, 1966-67. Natl. Mar. Fish. Serv., Biol. Lab., Seattle, Wash. Unpubl. manuscript.

January-February, June-July, and August 1967; and May 1968; and on a cruise of the MV *Paragon* in June 1966 (Table 1). Measurements in 1966-67 were south of Adak Island (long 176°25' W) except in January-February 1967 when the cruise track was along long 162° W and in May 1968 when it was along long 165° W (Figure 1). Primary productivity was measured by the carbon-14 method introduced by Steemann Nielsen (1952) and modified by Strickland and Parsons (1965). Productivity stations were normally taken shortly before dawn and local apparent noon (LAN); incubation periods were for one-half the daylight period, i.e., from dawn to LAN and from LAN until about twilight. Seawater was sampled with 6-liter plastic water bottles at depths determined from the penetration of light below the sea surface. These "light depths" were 100, 61, 35, 18, and 3% of the surface intensity according to the fractions of light transmitted by neutral-light filters used in the productivity incubations. The depths

TABLE 1.—Summary of areas, dates, and stations on which primary productivity was measured, 1966-68¹.

Cruise no.	Vessel	Dates	Number of stations	Area
1966				
K1-66	<i>Kelez</i>	March 18-28	Productivity - 6 Chlorophyll and nutrients - 9 Total - 9	Adak Is. to lat 41° N
P2-66	<i>Paragon</i>	June 10-21	Productivity - 8 Chlorophyll and nutrients - 10 Total - 10	Adak Is. to lat 41° N
K3-66	<i>Kelez</i>	Sept. 8-20	Productivity - 11 Chlorophyll and nutrients - 19 Surface productivity - 14 Total - 28	Adak Is. to lat 40° N
1967				
K1-67	<i>Kelez</i>	Jan. 30-Feb. 15	Productivity - 10 Total - 10	Along long 162° W between lat 54° and 46° N
K5-67	<i>Kelez</i>	June 24-July 3	Productivity - 10 Total - 10	Adak Is. to lat 46° N
² K6-67	<i>Kelez</i>	July 8-July 11	Productivity (1/2 day only) - 4	Alaskan Stream
K7-67	<i>Kelez</i>	Aug. 21-Aug. 28	Productivity - 7 Total - 7	Adak Is. to lat 46° N
1968				
K2-68	<i>Kelez</i>	May 9-15	Chlorophyll and nutrients - 5 Total - 5	Along long 164° W between lat 53° and 49° N

¹ Only portions of cruises discussed in this report are included in Table 1.

² Data from cruise 6-67 were averaged with data from station 5-67 for this report.

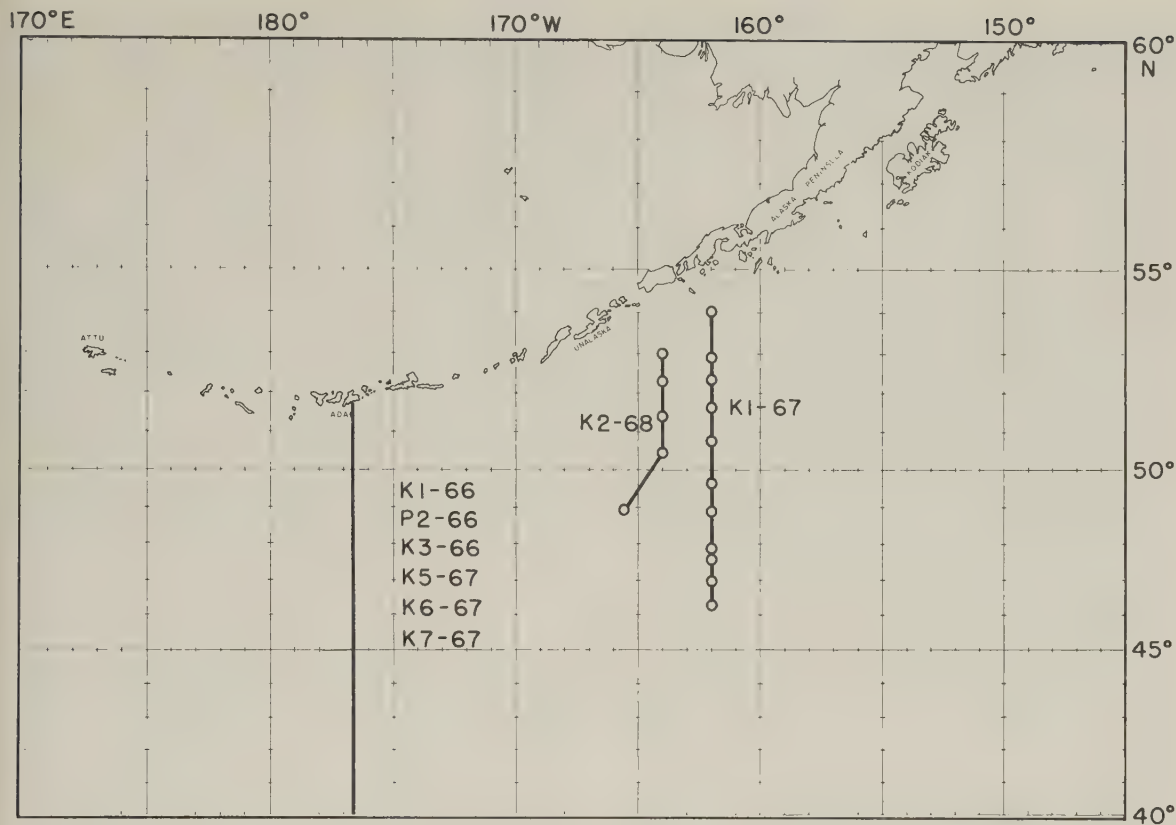


FIGURE 1.—Tracks of Bureau of Commercial Fisheries cruises in 1966-68 in the Subarctic Pacific Region during which primary productivity was measured.

were calculated from Secchi disk readings converted to extinction coefficients (Poole and Atkins, 1929). Sampling depths of the morning stations were computed from the preceding day's Secchi-disk readings. Duplicate light-bottle samples under the neutral-light filters were incubated on deck in daylight and cooled with running sea water. After incubation, samples were filtered through Millipore³ filters, pore size 0.45 μ , for radioassay.

Stock solutions of $\text{Na}_2^{14}\text{CO}_3$ were prepared according to Strickland and Parsons (1965) but were standardized by using liquid-scintillation techniques. A 1.00-ml portion from an ampoule

containing the stock carbonate solution was introduced in 10 ml of a suitable phosphor solution, and its count rate determined in a Packard Tri-Carb⁴ scintillation spectrometer. One liter of phosphor solution contained 800 ml toluene, 200 ml Sterox⁵ (a surfactant required to make the water miscible in toluene), 5.0 g PPO (2, 5-diphenyl-oxazole), and 0.3 g bis-MSB (p-bis-(o-methylstyryl)-benzene). Counts of an external radium standard were also recorded and the absolute activity (dpm) was determined from a quench correction curve relating efficiency to the count rate of the external standard (Wang and Willis, 1965). Although scintillation counting

³ Millipore Corp., Ashby Rd., Bedford, Mass. 01730. References to trade names in this publication do not imply endorsement of commercial products by the National Marine Fisheries Service.

⁴ Packard Instrument Co., Inc., 2200 Warrenville Rd., Downers Grove, Ill. 60515.

⁵ Jefferson Chemical Co., P.O. Box 53300, Houston, Tex.

is more efficient than planchet counting, productivity samples were routinely counted on planchets in a gas-flow geiger detector for convenience. The efficiency of the geiger counter was determined by counting a standard source of known activity so that absolute activity of the samples could be related to those of the stock solution.

Samples for chlorophyll *a*, phosphate, silicate, and nitrate-nitrite were also drawn from the water bottles. Nutrient samples were frozen in plastic bottles and returned to Seattle for analysis. Phosphate and silicate concentrations were determined by methods given by Strickland and Parsons (1965), and nitrate-nitrite samples were analyzed by the method of Wood, Armstrong, and Richards (1967).

Four liters of water from each sampler were filtered through glass-fiber filters (Gelman, type A),^{*} stored in a dark desiccator at about 0° C, and returned to Seattle for chlorophyll *a* analyses. A layer of MgCO₃ was added to the filter prior to filtration. Chlorophyll *a* concentrations were determined by the method of Richards with Thompson (1952) but were computed with equations given by Parsons and Strickland (1963). Chlorophyll samples on the glass-fiber filters were ground in a tissue grinder as suggested by Yentsch and Menzel (1963). The resulting suspension was filtered through a very-fine-porosity (VF) fritted-glass disk under pressure, and the cake of residue remaining on the disk was stirred with a few ml of 90% acetone and refiltered. Absorbances at 750 mμ, which indicate turbidity, of the resulting effluents were seldom more than 0.010 per cm of light path and then only in the more highly colored samples. A series of 20 tests showed no more than traces of pigment remaining in the residue after the first wash. The above treatment for separating residue from samples was preferable to centrifugation because it resulted in generally lower turbidity and more complete recovery of extract.

Total incident solar and sky radiation (over the wavelength range 0.3 to 3 μ) was continuously measured and graphically recorded by a

pyranometer. Although the photosynthetically active portion of the spectrum is roughly half the total radiation (Edmondson, 1956), total radiation values were used in productivity calculations. Salinity and temperature were measured and standard weather observations were recorded near the productivity stations (Larrance, 1971).

Measurements of productivity, chlorophyll *a*, and nutrients at various depths were integrated to the bottom of the euphotic zone (designated here as that depth where light intensity is 1% of the surface intensity), or other specified depth, and the integrated values expressed per square meter of sea surface. Mean values for several oceanographic domains were computed by weighting the values according to distances between stations. Details of the calculations were given in Larrance (1971).

PHYSICAL OCEANOGRAPHY

The physical oceanography of the Pacific Subarctic Region has been described by Fleming (1955), Dodimead, Favorite, and Hirano (1963), and Tully (1964). On the basis of data from Ocean Station "P" (lat 50° N, long 145° W), Dodimead et al. (1963) divided the upper 1,000 m of the Subarctic Pacific Region into three permanent zones: an upper zone from 0 to about 100 m depth; a halocline from about 100 to 200 m through which the salinity increases downward by about 1‰; and a lower zone from about 200 to 1,000 m. During the spring and summer, warming of the surface layers causes a temporary thermocline in the upper zone which is subsequently destroyed by cooling in the autumn. Consequently the lower limit of the wind-mixed upper layer ranges from about 30 to 60 m in the spring and summer and extends to the top of the permanent halocline at 100 m during winter. The upper zone in the Subarctic Pacific has been divided by Dodimead et al. (1963) into Coastal, Alaskan Stream, Central Subarctic, Western Subarctic, and Transitional Domains (Figure 2).

The Coastal Domain south of the Aleutian Islands lies over the continental shelf and is strongly influenced by Bering Sea water mixed

* Gelman Instrument Co., P.O. Box 1448, Ann Arbor, Mich. 48106.

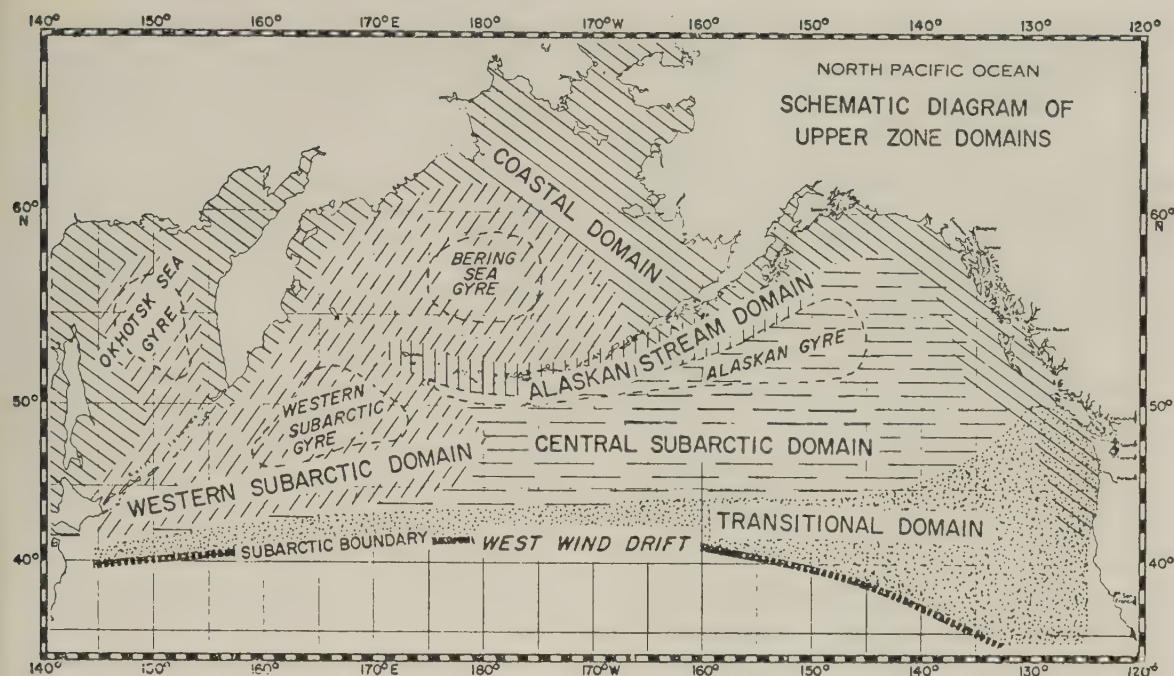


FIGURE 2.—Diagram of upper zone domains in the Subarctic Pacific Region (after Dodimead, Favorite, and Hirano, 1963).

through passes between the islands. To distinguish the Coastal from Alaskan Stream Domains, coastal water was arbitrarily defined by surface salinities greater than 32.9‰. The Alaskan Stream, described in detail by Favorite (1967), flows westward out of the Gulf of Alaska with velocities as high as 100 cm/sec. It is diluted by runoff from Alaska and can be detected by low salinity (less than 32.6‰) at the surface. The Central Subarctic Domain is an area of weak and variable currents bounded on the north by the Alaskan Stream and on the south by the Subarctic Current, which flows eastward at velocities between 5 and 20 cm/sec (McAlister et al., 1970). The Subarctic Current separates the Central Subarctic Domain from the Transitional Domain, which extends southward as far as the northern boundary of the Subtropical Region and is also an area of weak eastward flow. The nomenclature given by McAlister et al. (1970) was slightly different from that applied to the upper zone domains of Dodimead et al. (1963), but it was based in part on features

below the upper zone. For purposes of the present paper, the definitions of the upper zone domains as given in Dodimead et al. (1963) were used; the Subarctic Current, which originates in the Western Subarctic Domain, was thus included in the Central Subarctic Domain.

The Transitional Domain has been further divided into two areas (T-1 and T-2) on the basis of the salinity in the upper 50 m. The division between areas T-1 and T-2 was set in August 1967 at lat 47° N, where surface salinity was a maximum and decreased to the north and to the south at least as far as lat 46° N. The Transitional Domain was also divided in September 1966, when the northern area (T-1) extended from lat 47°05' N to a relatively sharp horizontal salinity gradient at lat 43°35' N. The southern area (T-2) extended to lat 40°45' N, where the boundary between transitional and subtropical waters was found. These divisions of the Transitional Domain are somewhat arbitrary but tend to be corroborated by biological and chemical characteristics.

PRIMARY PRODUCTIVITY ESTIMATES ADJUSTED FOR DIFFERENCES OF LIGHT INTENSITY

Because productivity was measured in natural light which differed (by as much as fivefold) in total insolation from day to day, productivity values were adjusted by two methods to permit comparison of productivity estimates under similar light conditions for purposes of detecting possible differences in productivity among oceanographic areas. One method applied the relation given by Ryther (1956) and Ryther and Yentsch (1957) of relative daily productivity beneath a unit of sea surface to total daily surface radiation. The measured daily light intensities were averaged for each cruise and the corresponding values of R (photosynthetic rate relative to photosynthesis at light saturation), defined by Ryther and Yentsch (1957), were determined for the cruise mean of daily light and for the light observed on the particular day in question (R_{av} and R_m , respectively). Adjusted productivity was then computed by $P_R = P_m \times R_{av}/R_m$ where P_R and P_m are the adjusted and observed productivities integrated through the euphotic zone and have the units of $\text{mg C}/\text{m}^2$ per day. This procedure amounts to using the shape of Ryther's (1956) curve but not his absolute values for estimating productivity.

Since Ryther derived his curve from photosynthesis measurements of phytoplankton from Woods Hole Harbor, that relationship is likely to differ from similar curves based on measurements from other areas. An attempt was made, therefore, to establish a simple empirical relation to estimate productivity in the mid-Subarctic Pacific Region from chlorophyll and light data obtained during the *Kelez* cruises. The regression of measured daily productivity (P_m) per unit of chlorophyll a (C_a) in the euphotic zone

$$\left(\text{where } P_m/C_a = \frac{\text{mg C assimilated}/\text{m}^2 \text{ per day}}{\text{mg chlorophyll } a/\text{m}^2}\right)$$

on daily light intensity measured on the ship's deck was computed (Figure 3). Only data from those stations where a full day's productivity and light were measured were used for the relation.

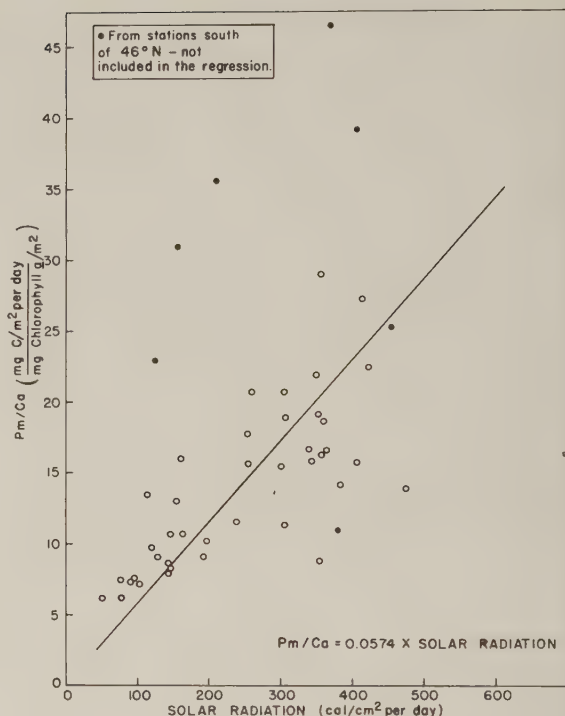


FIGURE 3.—Relation between the ratio of daily primary productivity to chlorophyll a (P_m/C_a) in the euphotic zone and daily solar radiation above the sea surface in the Subarctic Pacific Region, 1966-67.

The intercept of the regression and the axes was not significantly different from the origin. The variability was generally large, as might be expected from data of this kind, and was especially high for stations in the southern portion of the study area (transitional and subtropical water). This variability suggests that productivity responses to the environment in the Transitional Domain and subtropical water were probably different from those north of about 46° N. Furthermore, relatively few measurements were taken south of 46° N—not enough for seasonal comparisons. For these reasons, the regression was computed for only those stations north of 46° N.

Productivities under average daily light intensities for each cruise (P_R) were estimated by multiplying the chlorophyll a measured in the euphotic zone at a station by the estimate, from the regression, of P/C_a corresponding to the

average daily light intensity for the cruise. Although the relation was based on data taken north of lat 46° N, it was used to estimate productivity in June 1966 for stations as far south as lat 44° N (Figure 4).

These methods give rough approximations at best but probably indicate productivity responses to seasonal-average light conditions more accurately than those given by measured productivity values affected by large day-to-day fluctuations of light. The means of P_R for each cruise were higher than means of P_K except in September (Table 2, Figure 5). The higher P_R values may mean that the photosynthetic efficiency of the subarctic Pacific phytoplankton is lower than the average efficiency of populations represented in Ryther and Yentsch's (1957) analysis. Other possible explanations might involve differences between our experimental procedures and Ryther's (1956).

AREAL AND SEASONAL DISTRIBUTIONS OF PRIMARY PRODUCTIVITY AND CHLOROPHYLL *a*

Differences in productivity and chlorophyll *a* among the upper zone domains do not appear to be consistent from season to season except that mean values in the Coastal Domain and Adak Bay were higher than those farther from shore (Figure 4, Table 2). The Coastal and Alaskan Stream Domains can be compared only in June-July 1967 because both areas were not sampled on any other single cruise. Productivity and chlorophyll *a* were substantially higher in coastal water than in the Alaskan Stream. Unlike other times of the year, productivity and chlorophyll values were similar in nearshore and offshore areas in March 1966 and January-February 1967, probably because low light intensities in these months limited production to about similar levels throughout the northern Subarctic Region. This effect was especially pronounced at Adak Bay, where productivity estimates in March were between 350 and 460 mg C/m² per day but ranged between 840 and 2,400 mg C/m² per day in late spring and through the summer. The lowest productivity during each cruise was in the Central Subarctic Domain except in win-

ter, when productivity was uniformly low throughout the region. The mean productivity, however, was lower in the Central Subarctic Domain than in the other areas only in March 1966 and June 1967.

Daily carbon assimilation was normalized (P/C_a) above to estimate productivity from chlorophyll and light measurements. The commonly used P/C_a ratio may also be considered as an index of the capacity of a population to photosynthesize under natural light and ambient nutrient and temperature conditions, and provides a basis for seasonal and areal comparisons. This ratio is similar to "turnover rate" (Cushing et al., 1958) and to the ratio discussed by Currie (1958), except that Currie used the concentration of the total complement of plant pigments instead of only chlorophyll *a*. Platt (1969) used an efficiency index (productivity/light energy) to normalize productivity measurements for comparison at designated chlorophyll concentrations by means of a regression of the efficiency index on chlorophyll. His method was similar to that used here except he could estimate productivity at individual depths. For analysis in this study, ratios of P/C_a were computed for measured productivities (P_m) and productivities adjusted for differences in light (P_R) by the curve of Ryther and Yentsch (1957).

Ratios of P_R/C_a were high at four of the nine stations at or south of lat 46° N (Figure 6). The southernmost stations in March and September 1966 were in subtropical water. In March, P_R (515 mg C/m² per day) and P_R/C_a (39) were high, suggesting conditions of a phytoplankton bloom, whereas in September, P_R/C_a was lowest (10) of any observed during summer (Table 2). At lat 44° N in June 1966 and at lat $42^{\circ}50'$ N in September, P_R/C_a ratios were high (41 and 49), but P_R values were moderate (232 and 275 mg C/m² per day) and chlorophyll values were unusually low (5.6 mg/m² in each case). This combination of high P_R/C_a and low chlorophyll may be due to high carbon to chlorophyll ratios in the cells. At lat 46° N in August 1967, however, P_R and chlorophyll *a* were both relatively high (664 mg C/m² per day and 16.8 mg/m²); thus the high productivity and photosynthetic capacity ($P_R/C_a = 40$) of the popu-

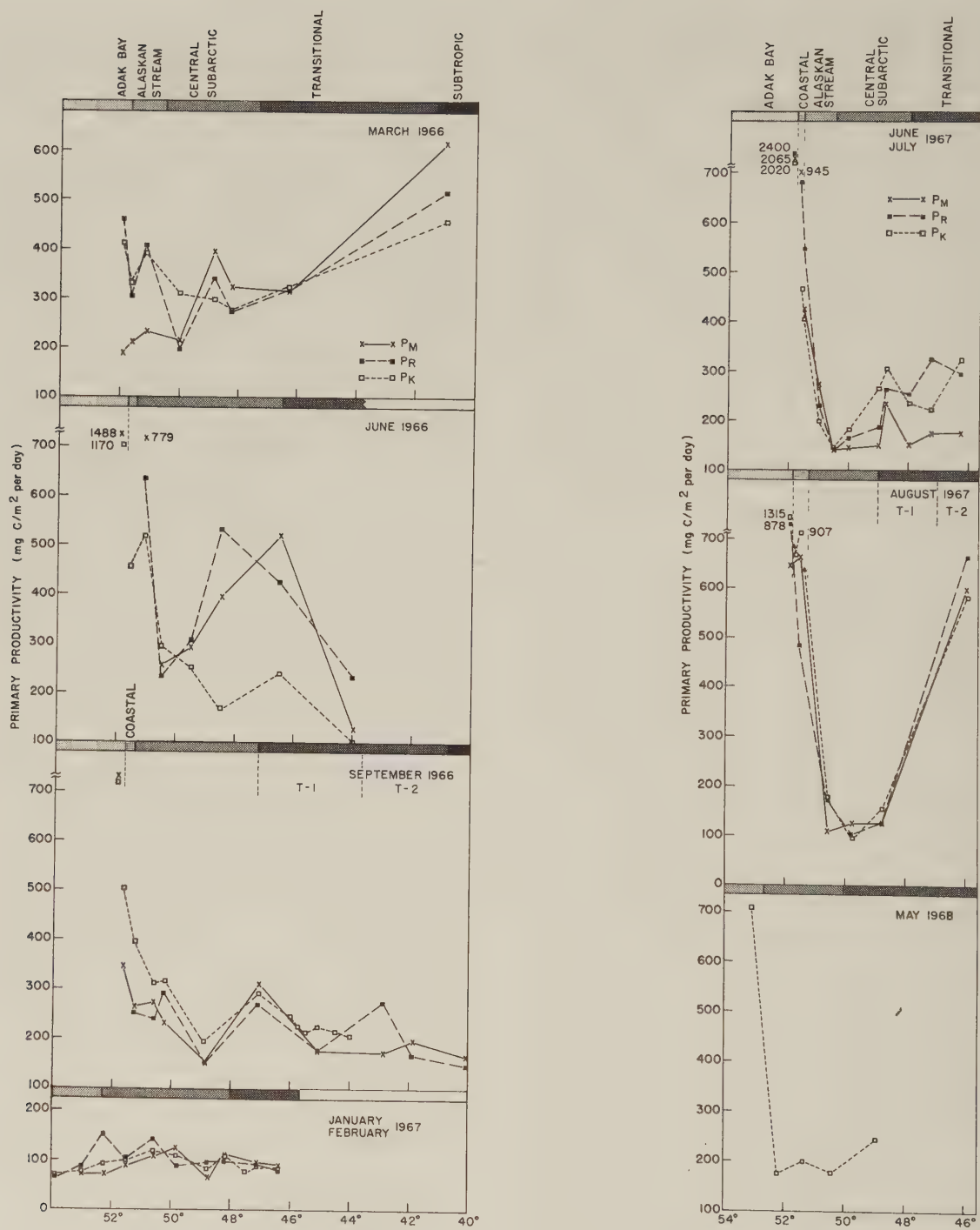


FIGURE 4.—Primary productivity in the mid-Subarctic Pacific Region, 1966-68.

TABLE 2.—Chlorophyll *a* in the euphotic zone, measured and estimated primary productivity values, and ratios of productivity to chlorophyll *a* in the mid-Subarctic Pacific Region (averaged within upper zone oceanographic domains) 1966-68.

Date and cruise no. and area	Chlorophyll <i>a</i> (mg/m ²)	Primary productivity (mg C/m ² per day)					
	<i>C_a</i>	<i>P_m</i>	<i>P_R</i>	<i>P_K</i>	<i>P_m/C_a</i>	<i>P_R/C_a</i>	<i>P_K/C_a</i> ¹
March 1966 (K1-66)							
Adak Bay	24.9	187	460	351	7.5	18.5	
Alaskan Stream	23.2	229	392	327	9.9	16.9	
Central Subarctic	17.8	305	265	251	17.1	14.9	
Transitional	19.6	317	317	276	16.2	16.2	
Subtropic	13.2	612	515	--	46.4	39.0	
46°-51°40'	19.3	292	303	272	15.1	15.7	14.1
June 1966 (P2-66)							
Adak Bay	64.3	1485	1492	1061	23.1	23.2	
Alaskan Stream	25.0	--	--	412	--	--	
Central Subarctic	14.5	422	429	239	29.1	29.6	
Transitional	9.4	324	328	155	34.5	34.9	
46°-51°40'	14.4	431	426	238	29.9	29.6	16.5
September 1966 (K3-66)							
Adak Bay	42.4	914	--	827	21.6	--	
Coastal	23.8	345	--	464	14.5	--	
Central Subarctic	11.9	195	199	232	16.4	16.7	
Transitional 1	11.7	222	197	228	19.0	16.8	
Transitional 2	6.9	187	217	--	27.1	31.4	
Subtropic	15.0	165	150	--	11.0	10.0	
46°-51°40'	12.8	204	201	250	15.9	15.7	19.5
January-February 1967 (K1-67)							
Alaskan Stream	9.1	71	80	60	7.8	8.8	
Central Subarctic	12.4	93	108	82	7.5	8.7	
Transitional	10.1	94	82	67	9.3	8.1	
46°-53°53'	11.2	88	96	74	7.9	8.6	6.6
June-July 1967 (K5-67 and K6-67)							
Adak Bay	99.4	2068	2396	1889	20.8	24.1	
Coastal	22.8	945	680	433	41.5	29.8	
Alaskan Stream	14.0	290	265	266	20.7	18.9	
Central Subarctic	11.1	165	193	211	14.9	17.4	
Transitional	13.2	171	299	251	13.0	22.6	
46°-51°40'	12.7	202	248	241	15.9	19.5	19.0
August 1967 (K7-67)							
Adak Bay	60.6	648	878	867	10.7	14.5	
Coastal	41.8	661	483	598	15.8	11.6	
Central Subarctic	6.3	118	138	90	18.7	21.9	
Transitional 1	7.2	127	127	103	17.6	17.7	
Transitional 2	16.8	598	664	240	35.6	39.5	
46°-51°40'	10.7	238	247	153	22.2	23.1	14.3
May 1968 (K2-68)							
Alaskan Stream	41.3			710			
Central Subarctic	10.6			182			
Transitional	14.3			246			
48°50'-53°	14.3			246			

¹ Mean values for each cruise.

lation were undoubtedly real. From these results, productivity and photosynthetic capacity south of lat 46° N appear to be neither characteristically high nor low, except in spring during bloom conditions, but fluctuate over a wide range.

Because relatively few measurements were made south of lat 46° N and the P_R/C_a ratios varied rather widely, only the data between lat

46° N and the Aleutian Islands were compared to determine seasonal patterns of production. If all the data are treated as if taken in the same calendar year, a seasonal pattern of productivity can be approximated (Figure 5). Productivity in winter was uniformly low throughout the area ($P_R = 96$ and $P_K = 74$ mg C/m² per day). The mean chlorophyll *a* concentration (11.2 mg/m²), however, was similar to

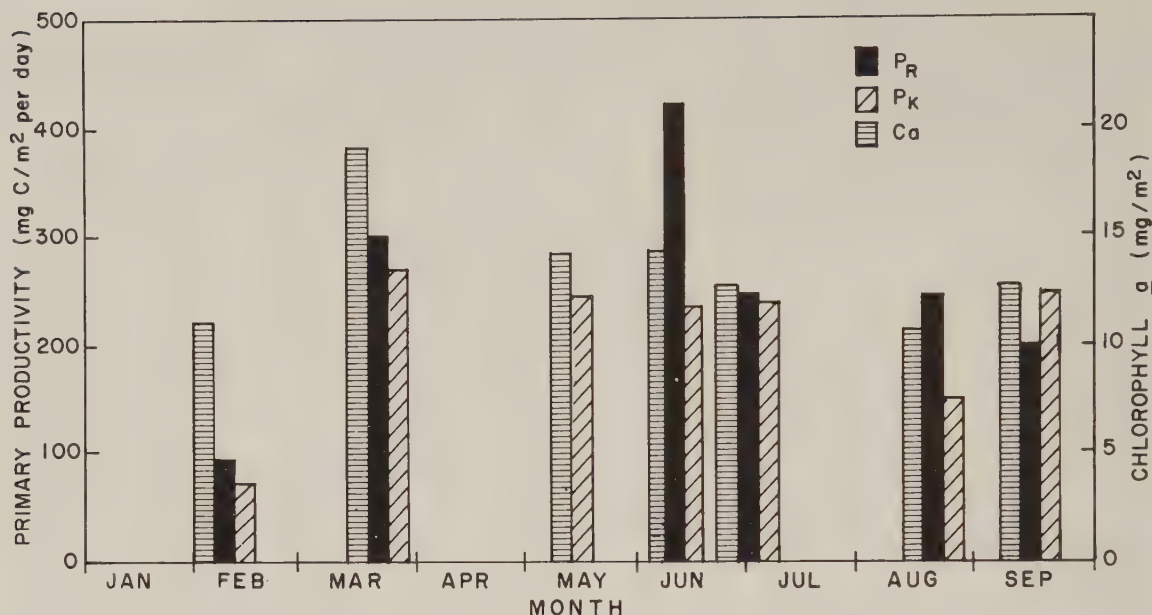


FIGURE 5.—Seasonal pattern of primary productivity and chlorophyll *a* in the mid-Subarctic Pacific Region between the Aleutian Islands and lat 46° N.

summer means. As might be expected, primary productivity was limited by low available light energy in the winter. The mean daily light intensity was low (116 cal/cm²), and the availability of light to the cells was further limited by their being distributed throughout the surface mixed layer, which reached well below the euphotic zone to the halocline at about 100 m.

In March, daily light intensities averaged 274 cal/cm² and thermal stratification had developed sufficiently to decrease the mixed-layer depth—thereby increasing exposure of the cells to light at shallower depths and consequently stimulating growth. The mean chlorophyll *a* concentration (19.3 mg/m²) was clearly higher than for any other season, and P_R and P_K (303 and 272 mg C/m² per day) were more than three times as high as in January and February (Figure 5, Table 2). The P_R/C_a and P_K/C_a ratios (16 and 14) were roughly twice those in midwinter, indicating that productivity was no longer limited by low light intensities. Although measurements in May 1968 were made at a considerable distance east of the Adak Line and may not be directly comparable, mean P_K values were re-

markably similar in June 1966 and 1967 and May 1968 (238, 241, and 246 mg C/m² per day, respectively) as were the mean chlorophyll values (14.4, 12.7, and 14.3 mg/m², respectively).

In August, the mean P_K and P_R values differed significantly ($P_K = 153$ and $P_R = 247$ mg C/m² per day). A large part of this difference may be attributed to high productivity measured at lat 46° N in transition area T-2 ($P_K = 240$ and $P_R = 664$ mg C/m² per day). These estimates strongly influenced the means because the weights assigned to each in the averaging process were relatively large. The mean chlorophyll *a* concentration (10.6 mg/m²) was slightly less than in June, and the mean P_R/C_a (23) was higher than in June. In September, mean P_R and P_K were 201 and 250 mg C/m² per day, and the mean chlorophyll *a* was 13.0 mg/m². These values were somewhat similar to the other summer values, as were the ratios P_R/C_a (16) and P_K/C_a (20).

The general time-distributional pattern of productivity and chlorophyll *a* between lat 46° and 51°40' N from January through September was drawn from the above results (Figure 5). Pro-

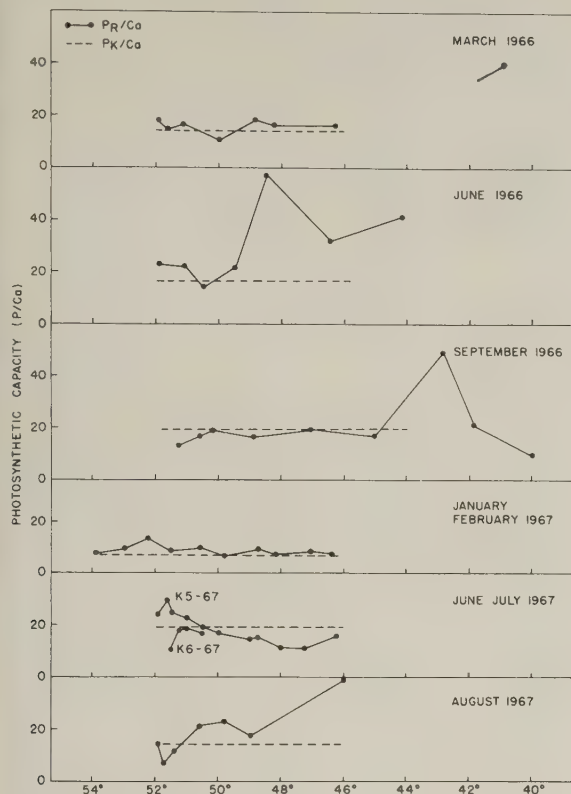


FIGURE 6.—Ratios of P_R/C_a and P_K/C_a in the Subarctic Region along long 175° - 176° W, 1966-67.

duction was low in the winter, increased significantly in March, and was relatively steady at intermediate levels throughout the summer. Chlorophyll *a* also increased between February and late March as a consequence of high production but decreased significantly during the spring and decreased slightly throughout the summer. Some reasons for these changes can be inferred from the nutrient and zooplankton data and are discussed later.

Although primary production apparently continued at a high rate through the spring, chlorophyll *a* concentrations were significantly less in May and June than in March. Two probable reasons for this decrease are a decrease in cell chlorophyll content and an increased loss rate, primarily due to grazing. It is impossible to ascertain from the data which one of these causes was most important, but some trial cal-

culations can help explore the problem. The standing stock (S_t) at t days (time between consecutive cruises) was predicted by the simple growth equation

$$S_t = S_0 e^{(a-b)t}$$

where S_0 is the initial standing stock and a and b are growth and loss coefficients. Standing stock was expressed in mg chlorophyll *a*/m², and P/C_a ratios were multiplied by $1/F$ (ratio of cell chlorophyll *a* to cell carbon) to give growth coefficients (a) in units of day⁻¹.

The growth rate (a) varied with time because P/C_a varied and $1/F$ was assigned values according to those reported elsewhere (Strickland, 1960; Eppley, 1968; and Strickland et al., 1969). Populations in nutrient-rich water under suboptimum light intensities, conditions extant in February and March, contain larger amounts of chlorophyll per unit carbon than those in nutrient-poor water under brighter light. Eppley (1968) found F values of about 30 for deep nutrient-rich water and 90 for shallow water depleted of nutrients. Values of $1/F$, therefore, were assumed to be 0.04 in February and March and 0.01 in June. The June value is probably too low because the water still contained ample nutrients (although lower than in March) for vigorous growth, but was selected to maximize the decrement afforded to decreasing cell chlorophyll content and therefore minimize S_t in June.

Results of the calculations show (Table 3) that changes in cell chlorophyll content could account for only part of the decrease in chlorophyll concentration between March and June. The loss coefficient (b) was computed for the period February to March and assumed to remain constant through June. The computed standing stock (S_t) in June was 1096, about 80 times as high as the observed value. (For comparison, standing stocks were also computed for $1/F = 0.04$ and $1/F = 0.01$, Table 3.) Since this (1096) is the minimum that could be expected from a loss of cell chlorophyll, grazing must have increased during the period to further decrease the chlorophyll concentration to its observed level. A concomitant increase in zooplankton corroborates this conclusion (discussed later).

TABLE 3.—Computed standing stock (S_t), expressed in units of chlorophyll, for various cell chlorophyll to carbon ratios ($1/F$) during spring 1966 in the mid-Subarctic Pacific Region.

Period	S_0	t	$1/F$	P/C_a	${}^1\bar{a}$	b	S_t
Feb.-Mar.	11.2	50	.04	7-15	0.433	±0.422	19.3
Mar.-June	19.3	80	.04-.01	15-20	0.472	0.422	±1096.
Mar.-June	19.3	80	.04	15-20	0.701	0.422	±9.3×10 ¹⁰
Mar.-June	19.3	80	.01	15-20	0.398	0.422	±28.0

¹ $\bar{a}$ is the mean growth coefficient calculated by assuming that P/C_a varied linearly with time as did $1/F$ in the second computation.

$$\bar{a} = a_0 + \frac{\Delta a}{t} \sum_{i=1}^{t-1} i$$

where $\Delta a = \Delta P/C_a [(1/F)_0 + 1/t \cdot \Delta 1/F] + (P/C_a)_0 \cdot \Delta 1/F$ and Δ is the difference between the initial and final values for the period.

² Computed values.

AREAL AND SEASONAL DISTRIBUTION OF NUTRIENTS

Nutrient concentrations were always higher in Adak Bay and the Coastal Domain than in the other areas and generally decreased toward the south (Table 4, Figure 7). Average concentrations of nutrients in the Central Subarctic water exceeded those in the Alaskan Stream only in June 1967. In winter of 1967, the nutrients were relatively high and varied little throughout the cruise area. Low nutrient concentrations at a few stations to the south in September correspond to lower P_R/C_a ratios. The lowest average phosphate concentration in the upper 50 m, 10 mg-at/m², was in subtropical water. Nitrate-nitrite was undetectable in the upper 10 m at one station in area T-2 (lat 43° N) and totaled only 12 mg-at/m² in the upper 50 m, whereas the minimum silicate observed was 187 mg-at/m² at the same station. Apart from the low nutrient values measured south of about lat 44° N in September and possibly in subtropical water in March, nutrients appeared to be in sufficient abundance to support vigorous phytoplankton growth. Even for these areas of low concentrations, productivity did not appear to be severely limited, as evidenced by the P_R/C_a ratios, but was probably somewhat suppressed.

To obtain a seasonal pattern of changes, the data on nutrients, like those on productivity, were treated as composite measurements from the same year. The changes of mean nutrient concentrations between lat 46° and 51°40' N from season to season were not large. In mid-

winter all nutrients were relatively abundant, as were phosphate and silicate in March. (Nitrate was not measured in March.) Phosphate decreased between March and June 1966 from 78 to 56 mg-at/m², the largest fractional change measured during this study for any of the nutrients. In June 1967, nitrate and phosphate concentrations were nearly equal to those in winter, but concentrations of silicate were lower. The apparent difference in the phosphate fluctuations between the 2 years could be the result of a shift in timing of the periods of high primary productivity, differences in supply by circulation, or an overall net difference in the balance between phosphate assimilation and supply for the year. By August phosphate and silicate had increased to quantities considerably higher than those in winter, but in September phosphate was lower and silicate was only slightly higher than in winter.

The low nutrient concentrations to the south in the summer lend support to the proposal by Anderson (1969) that a trans-Pacific band of chlorophyll occurs between the seasonal and permanent pycnoclines in the summer. His measurements indicate that the algae are produced in situ at these depths (50 to 75 m) which receive less than 1% of the light energy at the sea surface. This band lies between lat 35° and 45° N and is coincident with and could explain the occurrence of a layer of maximum oxygen content (Reid, 1962). Anderson also found that nitrate in the surface mixed layer above the chlorophyll band was nearly absent, having been used up in the spring during high primary production. A nitrate gradient through the deep

TABLE 4.—Dissolved nitrate, phosphate, and silicate in upper 50 m of mid-Subarctic Pacific Region (averaged within upper zone oceanographic domains), 1966-68.

Cruise no. and area	Nitrate mg-at/m ²	Phosphate mg-at/m ²	Silicate mg-at/m ²
K1-66			
Adak Bay	--	104.6	2,849
Alaskan Stream	--	88.8	1,990
Central Subarctic	--	81.2	1,903
Transitional	--	60.2	1,195
Subtropical	--	29.2	512
46°-51°40'	--	77.8	1,754
P2-66			
Adak Bay	--	94.9	2,871
Alaskan Stream	--	81.2	1,978
Central Subarctic	--	56.9	1,491
Transitional	--	49.6	1,155
46°-51°40'	--	56.0	1,480
K3-66			
Adak Bay	954	90.4	2,499
Coastal	--	88.7	2,331
Central Subarctic	583	75.4	1,692
Transitional I	492	56.7	1,143
Transitional II	64	16.8	280
Subtropical	76	10.0	335
46°-51°40'	579	72.0	1,597
K1-67			
Alaskan Stream	571	83.4	1,594
Central Subarctic	661	82.0	1,665
Transitional	599	69.0	1,314
46°-53°53'	627	79.0	1,561
K5-67			
Adak Bay	772	101.7	2,192
Coastal	1,160	117.4	2,806
Alaskan Stream	534	65.8	811
Central Subarctic	790	89.0	1,704
Transitional	248	42.2	588
46°-51°40'	579	78.6	1,247
K6-67			
Alaskan Stream	554	56.9	942
Central Subarctic	800	90.6	2,026
K7-67			
Adak Bay	1,111	113.6	3,012
Coastal	1,020	120.7	2,666
Central Subarctic	596	88.2	1,854
Transitional I	724	97.3	1,883
Transitional II	276	49.2	792
46°-51°40'	610	86.4	1,725
K2-68			
Alaskan Stream	--	60.0	868
Central Subarctic	--	63.1	1,022
Transitional	--	105.7	1,415

chlorophyll maximum suggested that nitrate diffused toward the surface from deeper water was completely assimilated in the deep layer. During the present study, chlorophyll maxima were found at 50 m in June 1966 at lat 44° N and at 100 m in September at lat 43° N. Nitrate was not measured in June but it was undetectable in

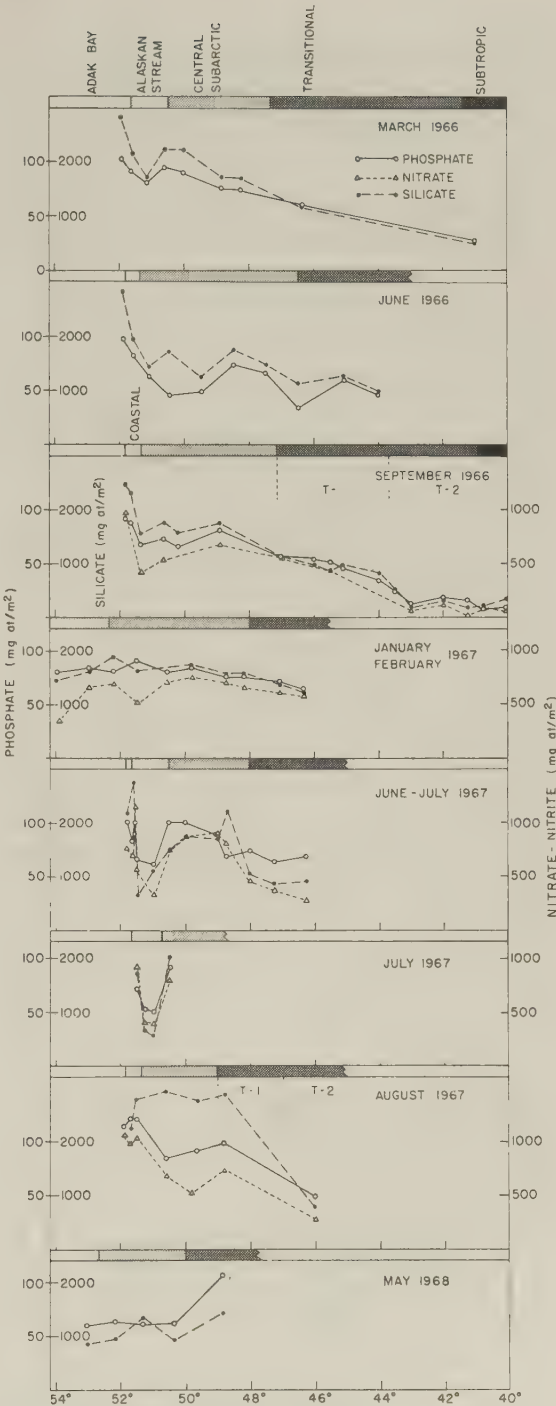


FIGURE 7.—Dissolved nitrate-nitrite, phosphate, and silicate in the upper 50 m of the mid-Subarctic Pacific Region, 1966-68.

the upper 10 m at lat 43° N in September. The deep chlorophyll layer at other stations could easily have been missed because sampling below the 1% light level was at standard depth.

PHOSPHATE CHANGES AND THEIR RELATION TO PRIMARY PRODUCTION

An attempt was made to draw qualitative inferences from phosphate data about the relative levels of primary production between cruises to obtain a somewhat more detailed picture of the seasonal productivity pattern. The major factors generally affecting changes in dissolved phosphate concentration in the oceans are: (1) utilization by primary producers (P_u), (2) regeneration by zooplankton and bacteria (P_r), and (3) advective changes (P_a). The relation among these factors is given by the formula:

$$P_0 = P_u + P_r + P_a,$$

where P_0 is the net change of phosphate concentration with time, P_u is negative, P_r is positive, and P_a can be of either sign. Estimates of P_u between cruises were calculated by applying a ratio of carbon to phosphorus (C:P) to the carbon-14 data as discussed below, P_a was estimated from calculations of vertical velocities and phosphate concentrations measured at depth, and P_0 was calculated from measurements of phosphate concentrations; however, no adequate estimate of P_r was possible. Two major assumptions were necessary to evaluate the above parameters:

1. The uptake ratio of C:P = 40 by weight (Strickland and Parsons, 1965). This value relates the C and P content of cells, but not the amounts assimilated. It is applied here, however, to carbon-assimilation rates measured by the carbon-14 method, which measures rates between net and gross production. The resulting estimate of phosphorus uptake, therefore, will be larger than the actual amount of phosphorus retained in new cell material.

2. Phosphate concentrations and their in situ changes were uniform within individual upper-zone domains. This assumption permits us to neglect the effect of horizontal advection.

Since nutrients were generally abundant in the Subarctic Region and changes were small during the year, circulation and regeneration must have supplied dissolved nutrients to the upper zone at rates sufficient to keep pace with their utilization, despite high assimilation rates by the algae during spring and summer. The amounts of phosphate supplied to the upper 50 m by upwelling were estimated from calculations of monthly mean vertical velocities (Wickett, 1966, 1968)⁷ and observed phosphate concentrations at 50 m (Table 5). Wickett listed meridional components of Ekman and total transport for alternate points on a grid of 5-degree units of latitude and longitude. To obtain the phosphate estimates, the average of monthly mean vertical velocities for grid points at lat 45° N, long 175° W and at lat 50° N, long 180° W were used as single monthly estimates applicable to the Adak line of stations north of lat 45° N. To obtain the net amount of phosphate exchanged through the 50-m surface, the net vertical displacement of water during each month was multiplied by mean phosphate concentrations at 50 m. The computed vertical velocities refer to the bottom of the Ekman layer which extends to the halocline at 100 m in winter but is limited by the thermocline in summer to as shallow as 30 m. The error incurred, however, by applying the velocities to 50 m rather than any other level was probably within the range of precision of the estimated velocities.

Such estimates of vertical transport of phosphate must be considered minimal, because the turbulent flux of properties across a surface cannot be computed by using mean velocities. That is, mean vertical velocities indicate net upward flow, although water and its associated properties actually move up and down across horizontal surfaces. When phosphate concentration increases with depth (as it usually does), the shallower water loses less phosphate by downward flux than it gains by equivalent up-

⁷ Wickett, W. P. 1966. Fofonoff transport computations for the North Pacific Ocean, 1966. Fish. Res. Board Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) 229, 92 p. (Processed.)

1968. Transport computations for the North Pacific Ocean, 1967. Fish. Res. Board Can., Tech. Rep. 53, 92 p. (Processed.)

TABLE 5.—Changes of dissolved phosphate in upper 50 m of mid-Subarctic Pacific Region (attributable to vertical transport and assimilation by phytoplankton) and their relation to measured concentrations (shown in parentheses).

Period	P_0 mg P/m ² per day (mg P/m ²)	P_a mg P/m ² per day (mg P/m ²)	P_u mg P/m ² per day (mg P/m ²)	Residual change of P^1 mg P/m ² per day (mg P/m ²)
1966				
March-early June	-8.1 (-680)	1.0 (80)	-6.4 to -9.3 (-530 to -760)	-2.7 to 0 (-230 to 0)
Late June-Sept.	5.4 (500)	1.5 (140)	-5.5 to -8.4 (-500 to -770)	9.4 to 12.3 (860 to 1130)
1967				
Feb.-June	0 (0)	2.7 (310)	-4.0 to -4.3 (-570 to -620)	1.3 to 1.6 (260 to 430)
July-Aug.	4.3 (240)	-1.7 (-100)	-4.9 to -6.2 (-280 to -350)	10.9 to 12.2 (620 to 690)

¹ See text for definition.

ward motion; the net upward flux of phosphate, therefore, is underestimated when mean vertical velocities are applied.

Values of P_u for the periods between cruises were estimated from productivity data. The lesser values of the two productivity estimates (P_K or P_R) from each of two succeeding cruises were averaged, as were the greater values. These averages represented the limits of the range of mean productivity during the period between cruises. For example, in the summer of 1966 (when in June, $P_K = 238$ and $P_R = 426$ mg C/m² per day, and in September, $P_K = 250$ and $P_R = 201$ mg C/m² per day), the range of mean productivity during the period was from 220 (the average of 238 and 201) to 338 (the average of 426 and 250) mg C/m² per day. The limits of the ranges were divided by the C:P ratio (40) to obtain the daily rate of phosphorus uptake in milligrams within a 1-m² cross-sectional column of the euphotic zone (Table 5). This rate was considered equivalent to the uptake in the upper 50 m. No error was incurred by this approximation when the euphotic zone was no deeper than 50 m. The "residual changes" of P were the changes unaccounted for by P_a and P_u ; thus they included regeneration, other changes not evaluated, and measurement errors:

$$\text{residual change} = P_0 - (P_a + P_u).$$

Although the accuracy of these estimates was low, the direction that P_a and P_u are likely to be in error is known and the direction of error of the

residual changes can be deduced. As the absolute values of P_a were minimal and those of P_u were too large (and P_a was either positive or negative and P_u was always negative), the sum $P_a + P_u$ tended to be underestimated. The residual changes, therefore, tended to be overestimated.

The residual changes during similar seasons in the 2 years indicate similar trends (Table 5). Negative values during spring 1966 show that phosphorus assimilation, and hence primary productivity, must have averaged more than that calculated, even if no regeneration occurred. If phosphate regeneration is assumed to be zero, the productivity during spring 1966 could have been as much as 40% higher than that calculated to account for the changes in measured phosphate. Although regeneration rates were probably lower than in summer, some regeneration probably occurred, and therefore the residual change would have been greater and the productivity even higher. Clearly, spring phytoplankton production in 1966 must have been substantially greater than the measured productivities.

Larger residual changes in the summer indicate higher phosphate regeneration rates than in spring. Phosphate turnover times ranging from about one to several months have been reported (Ketchum, 1962). According to Ketchum, excretion by zooplankton accounts for large portions of regenerated phosphate as well as inorganic nitrogenous compounds. The residual changes were correlated with zooplankton

biomass in the Subarctic Region, which was roughly three times higher in summer than in February or March (Donald S. Day, unpublished data).⁸ If phosphate regeneration accounted for all the residual changes in summer, 45 to 50% of the phosphate in the water would be renewed by regeneration in 3 months. In contrast, the upwelled phosphate supplied only about 6% of the total concentration in summer of 1966 and phosphate was lost from the upper layers in 1967 by mean velocities downward. According to the computed values (Table 4), however, in the summer the residual change of phosphate was roughly twice that removed from the water by plants. If the residual change is assumed to be mostly due to regeneration, therefore, the zooplankton would have had to release twice as much phosphorus as was taken up by the algae during the same period. A more likely explanation is that more phosphate was supplied from below than is indicated by the P_a values and the consequent residual changes would be less. In either case, in situ regeneration by zooplankton appears to be a major source of nutrients supplied throughout the summer in the mid-Subarctic Pacific Region.

At Ocean Station "P" primary productivity accounted for the entire loss of phosphate between March and August (Parsons, 1965),⁹ suggesting that regeneration was negligible. But, since zooplankton at Station "P" was sufficiently abundant to graze the phytoplankton to a stable level (McAllister, Parsons, and Strickland, 1960), it would seem that some phosphate regeneration should have occurred.

RELATION OF ZOOPLANKTON BIOMASS TO CHLOROPHYLL AND PRIMARY PRODUCTION

Data on zooplankton abundance and chlorophyll were compared to determine if these two

variables were correlated. The smaller zooplankters were sampled by raising a $\frac{1}{2}$ -m NOR-PAC net (mesh opening 0.33 mm) vertically from 150-m depth to the surface at about 1 m/sec. Displacement volumes of the catches weighted for distance between stations were averaged between lat 46° N and 51°40' N for each cruise. The mean volumes in February and March were about 0.070 ml/m³ of water strained and ranged from about 0.250 to 0.280 ml/m³ in summer, except in August 1967 when the mean volume was 0.550 ml/m³ (Day, see footnote 8). Thus, the zooplankton standing stock increased to about four times its winter level sometime after the phytoplankton increase in March. Grazing by the zooplankton apparently occurred early enough to crop down the algae, thereby limiting primary productivity before it reached sufficiently high levels to deplete the nutrients from the upper layers. A relatively steady state of grazing pressure and phytoplankton standing stock seemed to hold during the summer (at least in summer 1966). These findings agree with the conclusion of McAllister et al. (1960) that zooplankton grazing limited primary production at Ocean Station "P" by maintaining the phytoplankton standing stock at relatively low concentrations.

The relation between zooplankton displacement volumes and chlorophyll *a* concentrations (Figure 8) shows a negative correlation, further corroborating the above conclusion. The regression of chlorophyll *a* on zooplankton includes only those stations north of lat 46° N, except in coastal water and Adak Bay, and excludes all winter data. As shown previously the nearshore and transition waters exhibit chemical and biological features, which indicate ecological areas somewhat distinct from the area between. The winter data were also excluded from the regression because productivity was limited by insufficient light. Chlorophyll *a* concentrations south of lat 46° N showed no apparent relation to the amount of zooplankton present. Chlorophyll in Adak Bay and coastal waters was always significantly higher than estimated from the regression, except in March in Adak Bay. All of the high chlorophyll values were near shore and associated with intermediate quantities of zooplankton.

⁸ Donald S. Day, Oceanographer, Natl. Mar. Fish. Serv. Biol. Lab., Seattle, Wash.

⁹ Parsons, T. R. 1965. A general description of some factors governing primary production in the Strait of Georgia, Hecate Strait and Queen Charlotte Sound, and the N.E. Pacific Ocean. Fish. Res. Board Can., Manuscr. Rep. Ser. (Oceanogr. Limnol.) 193, 34 p. (Processed.)

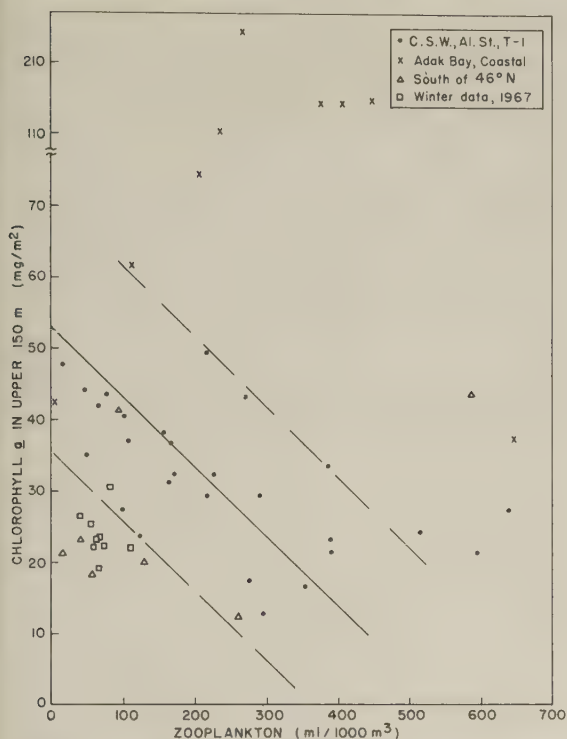


FIGURE 8.—Relation of chlorophyll *a* to net zooplankton in the upper 150 m of the Subarctic Pacific Region, 1966-68. Dashed lines represent 95% confidence limits.

The zooplankton value at the only nearshore station (Adak Bay) in March was the lowest observed. These data suggest that the zooplankton increase occurred later in the spring in coastal areas than farther offshore, a condition which could have permitted the phytoplankton bloom to reach much higher levels near shore before being controlled by grazing.

ANNUAL PRIMARY PRODUCTION

The annual primary production between lat 46° and $51^{\circ}40'$ N was estimated from values of P_K , P_R , and P_M . The data from all cruises were combined into a single composite year for apportioning productivity to time periods. The lowest estimate was for P_K (72 g C/m^2 per year); P_m and P_R were somewhat higher (82 and 85 g C/m^2 per year). If the estimate of primary productivity from phosphate changes is correct,

annual productivity could be as high as 100 g C/m^2 . These estimates are considerably higher than those reported by McAllister (1969), moderately higher than those of Koblents-Mishke (1965), and lower than those of Anderson (in press).

No reason is apparent from the quantitative data for the difference between annual productivity at station "P" (48 g C/m^2 per day; McAllister, 1969) and that in the mid-Subarctic Region. If zooplankton is the main factor limiting productivity, more zooplankton and less chlorophyll should be expected at station "P" than south of the Aleutians; however, zooplankton density was lower and chlorophyll *a* concentrations were about the same or slightly higher at station "P." More detailed seasonal observations from the mid-Subarctic, as well as a comparison of plankton communities in the two locations, will probably be necessary to explain the observed differences in annual productivity.

CONCLUSION

The main conclusions drawn from the primary productivity and related data are listed below:

1. No significant differences in primary productivity were found consistently among the various oceanographic domains or other waters of the Subarctic Pacific Region except that Adak Bay and the Coastal Domain south of Adak Island were generally more productive than areas farther south.

2. The annual cycle of productivity is typical for temperate oceans: productivity is low in winter, increases in the spring until more or less of a bloom develops, and declines by summer to relatively steady intermediate levels. Productivity in autumn and early winter was not studied.

3. Light limits productivity during winter; less light penetrates the surface than during other seasons, and the greater thickness of the mixed layer in the upper zone further reduces the light available to the algae.

4. During spring and summer, nutrients and light are plentiful, but zooplankton appears to graze the standing stock of phytoplankton to relatively low levels—thereby limiting productivity of phytoplankton.

5. The main mechanisms of dissolved-phosphate supply in the upper 50 m of water during summer are in situ regeneration by zooplankton and upwelling. Regeneration supplies significant amounts of phosphate (perhaps more than is provided by upwelling) in the summer, but increased turbulence and upwelling in winter maintain high levels of nutrients throughout the upper 100 m of water.

6. The seasonal cycle and factors limiting productivity are similar to those at station "P" (lat 50° N, long 145° W), but some significant differences exist. Productivity in the study area is nearly twice that at station "P," and zooplankton biomass is also much greater. The change in measured phosphate concentrations at station "P" over the productive season can be accounted for by phosphate utilized by the algae and by phosphate supplied by upwelling. South of the Aleutian Islands, however, phosphate regenerated in situ must be invoked to balance the phosphate budget.

7. Annual primary production in the area studied is about 80 to 100 g C/m².

ACKNOWLEDGMENTS

I am especially indebted to Thomas Elwell and Frederick Galassi for their technical assistance, particularly for their efforts at sea. I thank Dr. F. A. Richards for volunteering the services of chemists at the Department of Oceanography, University of Washington, to analyze the nitrate-nitrite samples. I wish also to express my appreciation to Dr. G. C. Anderson and Dr. Karl Banse, Department of Oceanography, University of Washington, for their many helpful criticisms of the manuscript.

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Iago, A NEW GENUS OF CARCHARHINID SHARKS, WITH A REDESCRIPTION OF *I. omanensis*

LEONARD J. V. COMPAGNO¹ AND STEWART SPRINGER²

ABSTRACT

A new genus, *Iago*, is proposed for *Eugaleus omanensis* Norman, 1939. *I. omanensis*, originally described from a single specimen, is redescribed from 16 additional specimens from the northern Arabian Sea continental shelf and slope between the Gulf of Oman and the Gulf of Kutch. Its presence in areas of low oxygen and the possibility of its occurrence in deeper waters of the Red Sea are discussed.

Norman (1939) described *Eugaleus omanensis* from a 280-mm female specimen, taken at 210-m depth in the Gulf of Oman. He placed it in *Eugaleus* Gill, 1864 (= *Galeorhinus* Blainville, 1816) with reservations because *omanensis* differed from all other species of *Eugaleus* in dentition and absence of a pronounced ventral caudal lobe. Norman noted that *omanensis* did not fit *Hemigaleus* Bleeker, 1852 because of dentition differences and lack of precaudal pits but he declined to establish a new genus for it.

Fowler (1941) overlooked *Eugaleus omanensis* in his review of Indo-Pacific elasmobranchs but later (1956) gave a description of the species condensed from Norman's account and allocated it to the genus *Galeorhinus*. Misra (1949) had earlier placed it in the same genus but this was not mentioned by Fowler.

Smith (1957) revised *Galeorhinus* but also overlooked *G. omanensis*. Compagno (1970) reviewed the systematics of *Hemitriakis*, *Galeorhinus*, and related genera. He considered *G. omanensis* generically distinct from *Galeorhinus* but did not propose a new genus in deference to this paper.

During the International Indian Ocean Expedition (IIOE) in 1963, the RV *Anton Bruun* on Cruise 4B conducted 109 trawling stations in transects along the continental shelf of the Arabian Sea between Bombay and the Gulf of Oman at depths from 15 to 375 m (Woods Hole Ocean-

ographic Institution, 1965). Sixteen specimens of a small carcharhinid were included in these collections and sent to us through the Smithsonian Oceanographic Sorting Center. They were tentatively identified by us as *Galeorhinus omanensis* (Norman).

Marshall and Bourne (1964, 1967), in photographic surveys of benthic fishes, collected 30 photographs of a small carcharhinoid shark (about 2 ft long) at depths between 1115 and 2195 m in the Red Sea. They noted that their shark might be either a triakid or a carcharhinid but was not identifiable to genus or species. Comparison of Marshall and Bourne's photographs and sketch of their "mystery shark" with our specimens and Norman's account of *G. omanensis* led us to suspect that the "mystery shark" might be *omanensis*.

We then sent two specimens of the IIOE series to Dr. N. B. Marshall at the British Museum (Natural History). At our request he compared them with the holotype, the hitherto only known specimen of *Galeorhinus omanensis*, and confirmed our identification of the IIOE specimens. He also agreed that the IIOE *omanensis* are very similar to the Red Sea "mystery shark" of the photographs, but noted that final identification of the Red Sea species must await capture of specimens.

Differences between "*Galeorhinus*" *omanensis* and members of *Galeorhinus*, *Hypogaleus*, *Hemitriakis*, and all other carcharhinid genera warrant the erection of a new genus for "*Galeorhinus*" *omanensis*.

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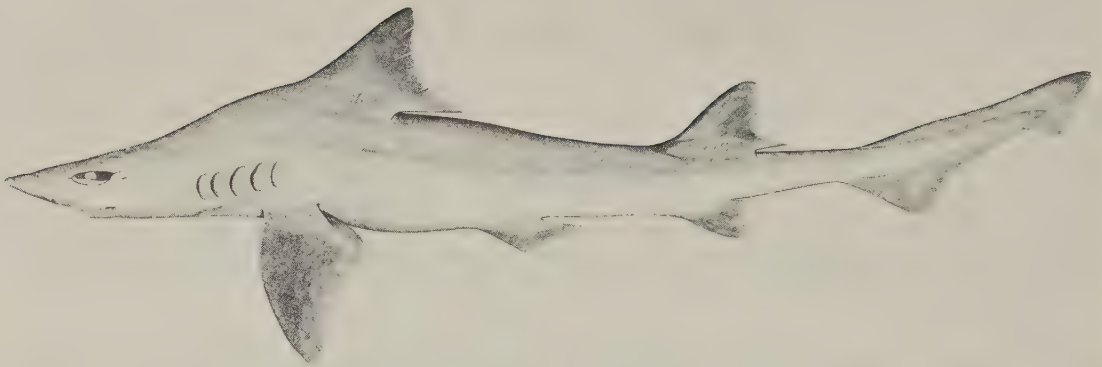


FIGURE 1.—*Iago omanensis*, from a 565-mm female deposited in U.S. National Museum. Drawing by Mildred H. Carrington.

Iago GENUS NOVUM

Eugaleus omanensis NORMAN, 1939,
TYPE-SPECIES

Etymology

This shark, a namesake of the villain of Shakespeare's *Othello*, is a troublemaker for systematists and hence a kind of villain.

Diagnosis (Terminology Follows Compagno, 1970)

Iago (Figure 1) differs from most carcharhinoids in the extremely anterior origin of its first dorsal fin. Only *Isogomphodon oxyrinchus*, a few species of *Carcharinus*, and the sphyrnid *Eusphyra blochii* rival *Iago* in this respect.

Iago is morphologically intermediate between the families Triakidae and Carcharhinidae as defined by Bigelow and Schroeder (1948) and Garrick and Schultz (1963). The characters of *Iago* strengthen the evidence presented by Compagno (1970) against separation of these families on simple nictitating lower eyelid and dental characters advocated by these writers.

We follow Compagno in uniting, at least provisionally, the two families. *Iago* thus falls into the family Carcharhinidae (sensu lato).

Iago is far from the advanced and intermediate carcharhinid genera discussed by Compagno (1970). These genera include *Hemigaleus*, *Hemipristis*, *Galeocерdo*, *Scoliodon*, *Rhizoprionodon*, *Loxodon*, *Negaprion*, *Triaenodon*, *Lamiopsis*, *Isogomphodon*, *Carcharhinus*, *Hypoprion*, and *Aprionodon*. *Iago* differs from all of these in having a transitional, not internal, nictitating lower eyelid with edge nearly horizontal; shallow subocular pouch; teeth with strong basal ledges and grooves; teeth at symphysis only slightly smaller than adjacent ones; no precaudal pits; pectoral fin skeleton projecting less than halfway into fin; distal pectoral radials only as long as proximals, with parallel edges and truncate tips (not tapered and acute); caudal fin without projecting ventral lobe and lateral undulations of its dorsal margin in adults; cranium with a complete supraorbital crest (absent in advanced forms); and a spiral, not scroll, intestinal valve (*Hemigaleus* and *Hemipristis* are exceptional in also having spiral valves).

Iago differs from *Galeorhinus* and *Hypogaleus* as delimited by Compagno (1970) in having a transitional rather than internal nictitating low-

er eyelid in adults, with nearly horizontal edge; anterior nasal flap not greatly reduced; teeth without postlateral cusplets; an interdorsal ridge present; lateral trunk denticles much longer than wide in adults (about as long as wide in *Hypogaleus* and *Galeorhinus*); and no ventral lobe on caudals of adults.

From the curious *Leptocharias*, *Iago* differs in having a transitional rather than internal nictitating lower eyelid in adults; much larger spiracles; no nasal barbel; very weak gynandric heterodonty; teeth with primary cusps oblique, not erect, and lacking cusplets; sharp-edged, bladelike cutting teeth; fewer total vertebrae, 130 to 147 (198 to 214 in *Leptocharias*); spiral intestinal valve with about 5 turns (14 to 16 in *Leptocharias*); and an entire supraorbital crest (reduced in *Leptocharias* to isolated preorbital and postorbital processes).

Iago can be distinguished from *Hemitriakis*, *Furgaleus*, *Scylliogaleus*, the *Triakis-Mustelus* complex, *Proscyllium*, and *Eridacnis* by its more lateral eyes, in dorsal view nearly touching head rim, and its sharp-edged, monocuspidate teeth. In addition, *Iago* differs from *Hemitriakis* in having a transitional rather than external nictitating lower eyelid, noncarinate posterior teeth, more tooth rows (only 18 to 36/29 to 34 in *Hemitriakis*), weak transverse notches on teeth, and no ventral caudal lobe. *Iago* lacks the short, thick, rounded snout, nasoral grooves, and molariform teeth of *Scylliogaleus* and also has fewer tooth rows and series of teeth functional. Unlike *Furgaleus*, *Iago* lacks nasal barbels, erect cusps on its lower anterolateral teeth, and a ventral caudal lobe; also, *Iago* (Figure 2) has the nostrils definitely closer to the mouth than the snout tip (about equidistant in *Furgaleus*). *Iago* differs from most members of the *Triakis-Mustelus* complex in having fewer tooth rows and series of teeth functional; however, *Triakis semifasciata* rivals *Iago* in these respects. *Iago* does not have a pavement of molariform teeth as in *Mustelus*; also, its pelvic anterior margins are less than half the length of pectoral anterior margins (over half as long in *Triakis-Mustelus*). Finally *Iago* contrasts with *Proscyllium* and *Eridacnis* by its transitional, not rudimentary, nictitating lower eyelid; monocuspidate poster-

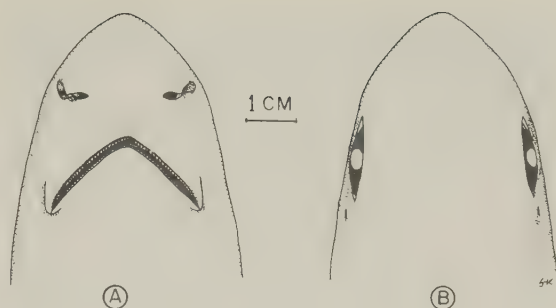


FIGURE 2.—*Iago omanensis*. A. Ventral side of head. B. Dorsal side of head.

ior teeth (not comblike); dorsal fin base midpoint closer to pectoral base termination than pelvic origins (vice-versa in *Proscyllium* and *Eridacnis*); second dorsal origin anterior (not over or posterior) to anal fin origin; intermediate of vertebral centra strong wedges (not wedgelike in *Proscyllium* and *Eridacnis*); large papillae absent from gill arches and buccal cavity; and nostrils farther apart. *Iago* also lacks the clasper hooks, scylliorhinoid color pattern, and apparently the oviparous reproduction of *Proscyllium*.

GENERIC DESCRIPTION

Head flattened, its length from snout tip to fifth gill opening about $\frac{1}{4}$ total length.

Eye openings dorso-lateral, not visible in ventral view of head, openings elongate, about twice as long as high, with a well-developed posterior notch; nictitating lower eyelid transitional (Figure 3B), its edge nearly horizontal; secondary lower eyelid strongly differentiated, its edge thin; subocular pouch shallow, its lateral surface bare of denticles.

Slitlike spiracles, length about $\frac{1}{7}$ eye length, located about $\frac{2}{7}$ eye length behind and slightly below posterior eye notch; external gill slits moderately short, lengths in adults nearly equal, the longest $\frac{1}{2}$ to $\frac{2}{3}$ eye length; nostrils located about one-half as far from mouth as from snout tip, well separated, without nasoral grooves, widths about $1\frac{2}{3}$ to 2 times internarial distance, anterior nasal flap a short truncate lobe.

Mouth opening subtriangular, broad, 2 to $2\frac{1}{2}$ times as wide as long; labial furrows extending around mouth corners, the upper furrows longer, extending anteriorly only to below eye pupils; large papillae absent from buccal cavity.

Teeth small (Figures 3A and 4), largest with greatest width at root about 1.5 mm in 457-mm female; tooth rows 46 to 55/37 to 45; 2 to 3 series functional along edges of jaws; teeth in mixed alternate and imbricate overlap pattern of Strasburg (1963); no serrations; premedial edge of crown in anteroposteriors convex, post-lateral edge deeply notched forming a low post-lateral blade on crown foot; all teeth with a strong basal ledge and groove, transverse ridges on crown foot; roots low, deep, with transverse



FIGURE 4.—*Iago omanensis*. Teeth of right side of upper and lower jaws; labial aspect. Dotted lines indicate jaw symphysis.

primary cusp and no cusplets; anteroposteriors show moderate gradient monognathic heterodonty, with teeth becoming smaller, more oblique-cusped, and lower-crowned towards ends of dental band; posteriormost teeth with strong primary cusps; ontogenic heterodonty not known at present; gynandric heterodonty indicated only by slightly more erect cusp tips on anteroposteriors of adult males.

Trunk not markedly compressed, less than twice as high as wide, subtriangular in cross section; a low interdorsal ridge present; lateral dermal keels and precaudal pits absent from caudal peduncle.

Dermal denticles of trunk below first dorsal longer than wide, crown with a high, narrow ridge extending to tip of posteriorly directed cusp; a pair of lateral ridges weakly developed or absent, lateral cusps weak or absent.

Pectoral fins larger than first dorsal fin in area, their anterior margins about $1\frac{1}{2}$ times as long as combined base and inner margin lengths; distal tip of adpressed pectoral about over its free rear tip when pectoral inner margin is held parallel to body axis; origin of pectoral below or slightly in advance of fourth gill opening; pectoral skeleton projecting less than halfway into fin, its longest distal radials about equal in length to corresponding proximal ones; distal radials with truncate tips and parallel edges.

Pelvic anterior margins less than half length of pectoral anterior margins; pelvic bases equidistant between first and second dorsal bases.

Claspers with pseudoparae, pseudosiphons, cover rhipidia, true rhipidia, and exorhipidia (Figures 5B and 5C); siphon sacs large, extending anteriorly to level of pectoral free rear tips (Figure 5A); margins of clasper cartilage rolled, with margins overlapping to form a tube; clasper hooks absent.

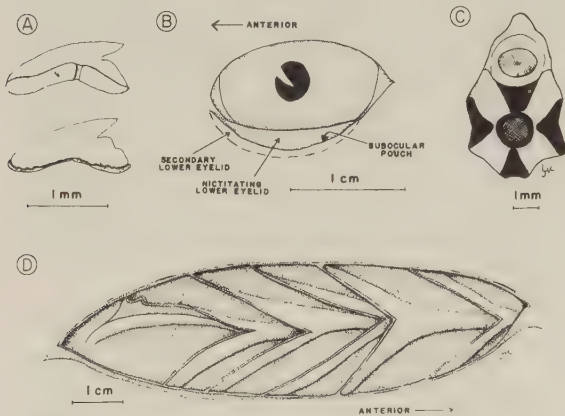


FIGURE 3.—*Iago omanensis*. A. Typical teeth: upper, buccal surface; lower, labial surface. B. Eye. C. Cross section of trunk vertebra; thoroughly calcified areas shown in black. D. Valvular intestine, one side cut away. Drawing by L. J. V. Compagno.

groove on attachment surface but transverse notch weak; teeth not noticeably protruding when mouth is closed.

Dignathic heterodonty very weak, with upper anteroposteriors having slightly higher crowns than lower ones; disjunct monognathic heterodonty indicated by differentiation of medials in one row on upper jaw and about 3 in lower; medials smaller, with erect primary cusps, large premedial and postlateral blades, and no cusplets; larger anteroposteriors are sharp-edged, compressed, bladelike cutting teeth with an oblique

Origin of first dorsal fin far forward, varying in position from above fourth gill opening to slightly before pectoral axilla; midpoint of first dorsal base much closer to pectoral axilla than to pelvic origins; free rear tip of first dorsal anterior to pelvic fin origins.

Second dorsal nearly as large as first, its height about 70% of first dorsal height; its posterior margin strongly concave.

Anal smaller than second dorsal, slightly more than half its height, its base about $\frac{2}{3}$ of second dorsal base length; its posterior margin nearly straight or shallowly concave; its origin posterior to second dorsal origin by about $\frac{1}{5}$ to $\frac{1}{2}$ of second dorsal base length; posterior ends of second dorsal and anal bases opposite.

Caudal without projecting ventral lobe-tip in adults, preterminal margin slightly more than $\frac{1}{3}$ of dorsal margin length; subterminal margin long, over half length of terminal margin; caudal dorsal margin length about $\frac{1}{5}$ of total length; terminal sector of caudal about $\frac{1}{3}$ of dorsal margin length; vertebral axis of caudal only slightly raised above body axis.

Vertebrae moderately numerous, 129 to 147 in total count ($N = 16$). Monospondylous precaudal (MP) centra 24.5 to 27.6% of total count; diplospondylous precaudal (DP) centra 33.6 to 36.1; and diplospondylous caudal (DC) centra 37.2 to 40.1 ($N = 8$). A ratios 120 to 162, B ratios 102 to 137 ($N = 11$). DP and DC centra more numerous than MP centra and nearly equal to each other, DP/MP ratio 1.22 to 1.46 and DC/MP ratio 1.38 to 1.63 ($N = 8$). Transition between MP and DP centra easily delimited on radiographs, over pelvic region. Posteriormost MP centra not greatly hypertrophied. DP centra of relatively uniform length throughout, not forming a stutter zone of alternating long and short centra.

Vertebral calcification pattern a modified version of White's (1937) "Maltese cross" pattern, without diagonal calcified lamellae; notochordal canal unusually large (Figure 3C); wedgelike intermedialia strongly developed.

Supraorbital crest of cranium strongly developed and entire.

Intestinal valve of spiral type, with about five turns.

Iago is apparently livebearing (see section in Reproduction below), but whether or not a yolk-sac placenta is formed cannot be determined from available specimens.

Iago omanensis (NORMAN, 1939)

Eugaleus omanensis Norman, 1939, p. 11, Fig. 3 (type-locality, Gulf of Oman); Compagno, 1970 (generic systematics).

Galeorhinus omanensis Misra, 1949, p. 21 (in list of Indian elasmobranchs, name only); Fowler, 1956, p. 17 (description, after Norman); 1967, p. 363 (in list of fishes of the world, name only).

MATERIAL

Seven males, 224 to 365 mm; nine females, 358 to 582 mm (Table 1); holotype, British Museum (Natural History) Reg. No. 1939.5.24.9, a 280-mm female from Gulf of Oman. Specimens

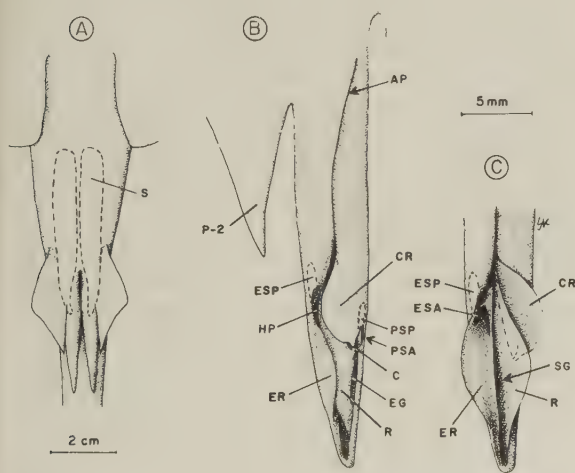


FIGURE 5.—*Iago omanensis*. A. Ventral aspect of trunk of mature male to show size and position of clasper siphons. B. Dorsal surface of left clasper. C. Partially expanded tip of left clasper. AP, apophysis; C, connection between rhipidion and cover rhipidion; CR, cover rhipidion; EG, epirhipidial groove; ER, exorhipidion; HP, hypopygium; P-2, pelvic fin; ESA, pseudoperaperture; ESP, pseudoperaperture; P-2, pelvic fin; PSA, pseudoperaperture; P-2, pelvic fin; R, rhipidion; S, siphon sac; SG, subrhipidial groove.

TABLE 1.—Oceanographic data for *Iago omanensis* from Final Cruise Report, Anton Bruun Cruise 4B, Woods Hole Oceanographic Institution (1965).

Length of specimen	Sex	IIOE station number	Date	Lat N	Long E	Bottom temperature	O ₂ at bottom	Depth
mm						° C	ml/liter	m
312	♂	227A	11/19/63	22°38'	67°11'	22.39	0.77	110
224	♂	231A	11/20/63	23°13'	66°40'	19.89	0.24	183-155
457	♂	255A	11/30/63	25°50'	57°07'	24.47	2.40	92-95
427	♀	255A	11/30/63	25°50'	57°07'	24.47	2.40	92-95
440	♀	264A	12/02/63	25°02'	56°52'	20.02	0.60	291-272
480	♀	264A	12/02/63	25°02'	56°52'	20.02	0.60	291-272
472	♀	264A	12/02/63	25°02'	56°52'	20.02	0.60	291-272
363	♂	264A	12/02/63	25°02'	56°52'	20.02	0.60	291-272
358	♀	268A	12/03/63	24°12'	57°26'	16.24	0.28	364-368
565	♀	268A	12/03/63	24°12'	57°26'	16.24	0.28	364-368
582	♀	268A	12/03/63	24°12'	57°26'	16.24	0.28	364-368
335	♂	268A	12/03/63	24°12'	57°26'	16.24	0.28	364-368
365	♂	268A	12/03/63	24°12'	57°26'	16.24	0.28	364-368
395	♀	279B	12/09/63	24°13'	65°52'	19.34	0.22	170-192
304	♂	279B	12/09/63	24°13'	65°52'	19.34	0.22	170-192
295	♂	279B	12/09/63	24°13'	65°52'	19.34	0.22	170-192

mens are deposited in the collections of the U.S. National Museum, California Academy of Sciences, and the British Museum (Natural History).

DESCRIPTION

Proportional dimensions as percentages of total length are given for our 16 specimens in Table 2.

In lateral view head outline slightly convex dorsally and ventrally, its outline tapering smoothly to snout tip; outline of head in dorsal view parabolic in shape, the sides converging at a narrow angle from gill openings to nostrils but then converging at a much wider angle to snout tip; head broad, width at spiracles about half its length; interocular slightly less than width of head at eyes; head subquadrate in transverse section at eye pupils.

Snout tip narrowly rounded in dorsal view, bluntly pointed in lateral view; preoral length 1.1 to 1.4 in mouth width.

Eye length equal to or slightly longer than distance between nasal apertures; eye openings subelliptical, rounded anteriorly.

Nictitating lower eyelid (NLE) a variety of Compagno's (1970) transitional type, intermediate in form between "nictitating folds" and "nictitating membranes" of authors (Figure 2B); its anterior edge smoothly confluent with

edge of upper eyelid; secondary lower eyelid (SLE) originating beneath origin of NLE; posteriorly NLE merges with SLE and continues as single edge to notch; subocular pouch or pocket between NLE and SLE extends beneath anterior two-thirds of eye; SLE edge sharp and thin with NLE is artificially raised to the limits of its travel; dermal denticles present on outer face of NLE. The structure and the kinetics of the NLE in *Iago omanensis* suggest that it cannot cover the posterior third of the eye when raised as it can in adults of *Galeorhinus* and higher carcharhinoids.

Spiracles open below level of posterior eye notch and are posterior to eye by distance about equal to their own length.

Gill openings increasing in size from first to third by small increments, with fourth slightly shorter than third and fifth about half length of fourth, becoming more dorsally situated from anterior to posterior, with upper origin of first opposite midpoint of fifth. Definite gill rakers not developed, but small dermal mounds are present on gill arches.

Nostrils relatively large, their openings obliquely directed anterolaterad; anterior nasal flap varying from subtriangular to lobate; a small inner nasal flap present dorsal to anterior nasal flap and concealed by it; posterior nasal margin with an elongated process (stiffened by ala nasalis) that projects obliquely dorsomedially into nasal cavity.

TABLE 2.—Proportional measurements of *Iago omanensis* expressed as percentages of total lengths; measurement method follows Bigelow and Schroeder (1948).

	Male 335 mm	Range in 7 males	Female 565 mm	Range in 9 females
Tip of snout to:				
Front of mouth	7.2	6.1-7.6	5.8	5.7-6.4
First gill opening	19.1	17.4-20.0	16.8	16.2-17.8
Last gill opening	24.2	24.2-27.5	23.4	23.0-24.5
Origin pectoral fin	23.3	23.3-25.9	22.7	21.2-23.5
Origin first dorsal fin	25.9	25.3-29.5	24.8	24.0-27.1
Origin pelvic fins	42.8	41.6-45.9	43.9	42.7-46.5
Origin second dorsal fin	56.8	56.8-60.6	58.4	57.8-59.9
Origin anal fin	58.3	58.3-63.4	59.2	59.2-63.3
Anus	44.7	43.5-49.1	45.1	44.5-47.4
Distance between fin base:				
First and second dorsals	23.5	21.4-25.3	24.2	21.9-24.6
Pectoral and pelvic	16.1	13.7-16.1	18.6	16.8-20.0
Pelvic and anal	11.0	11.0-12.3	11.5	11.0-13.2
Anal and lower caudal	8.9	7.2-10.5	8.5	7.5-9.7
Length eye opening	5.1	4.2-5.1	4.1	3.3-4.1
Least internasal distance	4.0	3.6-4.2	3.7	3.3-3.9
Width mouth	7.2	7.7-9.4	7.1	6.4-8.4
Length upper labial furrow	1.5	1.5-2.0	1.2	1.1-1.2
Length lower labial furrow	0.9	0.8-1.4	0.9	0.6-1.1
Diameter spiracle	0.6	0.4-0.8	0.5	0.2-0.6
Distance, spiracle to eye	0.7	¹ 0.7-1.1	1.1	¹ 0.7-1.2
Fin measurements:				
Base first dorsal	8.9	8.9-11.2	9.4	8.3-11.6
Height first dorsal	6.0	6.0-7.8	7.8	7.0-8.8
Base second dorsal	8.9	8.0-9.7	7.3	7.3-9.1
Height second dorsal	4.8	4.5-6.1	5.5	5.2-6.5
Base anal	6.0	5.5-6.6	5.3	4.9-6.4
Height anal	3.0	2.6-3.4	3.5	2.8-4.0
Width pectoral base	4.8	4.7-5.6	4.8	4.5-5.6
Anterior margin pectoral	14.3	12.7-14.3	15.3	13.4-16.6
Overall length pelvis	8.6	8.3-9.9	9.6	8.5-9.6
Upper margin caudal	22.4	20.1-22.4	21.9	20.8-22.7

¹ In 4 males or 6 females.

Mouth width about two-thirds of width of head at mouth corners; edge of lower jaw convex.

Teeth show modest gradient heterodonty; from symphysis to mouth corner teeth become lower relative to their root lengths; tooth size changes also along this gradient, starting from small to largest in about four rows from medials and gradually becoming smaller towards ends of dental band.

Body moderately slender, trunk rather high anteriorly, almost humped above pectorals, sloping posteriorly to pelvis and caudal origins; caudal peduncle long, slender, subquadrate in cross section, a weak postdorsal ridge extending medially from just after second dorsal to caudal origin; an ill-defined predorsal ridge extending for a short distance anterior to first dorsal fin; distance from snout tip to cloaca somewhat less than distance from cloaca to caudal tip.

Dermal denticles small, those of dorsal surface below first dorsal fin base 0.04 to 0.08% of total length in two specimens, 0.15 to 0.35 mm in 457-mm female; anterior edges and posterior cusps of adjacent denticles somewhat overlapping, skin visible between denticles; denticles transparent, without pigment and more or less invisible when wet; chromatophores of skin between denticle bases visible through denticles; bases of denticles short, subquadrate, with relatively short pedicels; medial ridge of crown not subdivided longitudinally (Figure 6); concave depression present on either side of medial ridge, with its lateral boundary deflected slightly outward to form an incipient lateral ridge that may or may not terminate in a short lateral cusp; lateral cusps generally absent from denticles of caudal and ventral surfaces; denticles below first dorsal becoming wider relative to their lengths with increase in specimen size; small denticles



FIGURE 6.—*Iago omanensis*. Dermal denticles from side of trunk below first dorsal fin, 582-mm female, scanning electron microscope photomicrograph, scale line at lower left equals 100 μ .

(less than 0.1 mm long) present on anterior part of palate and inner surfaces of branchial arches, also irregularly and rather sparsely scattered on tongue.

Pectoral fins broad, subangular, with their anterior margins convex, apices rounded, posterior margins slightly convex, free rear tips rounded, and inner margins convex, relatively short, their anterior margins about an eye diameter shorter than distance from snout tips to first gill opening; pectoral inner margins long, about $1\frac{1}{2}$ to $1\frac{1}{3}$ times pectoral base length; posterior margin about $1\frac{1}{3}$ to $1\frac{1}{2}$ times in anterior margin; free rear tip of pectoral varying in position from below posterior end of first dorsal base to last third of first dorsal base.

Pectoral fin skeleton, as studied on radiographs, somewhat similar to that of *Galeorhinus*; propterygium with 1 radial, mesopterygium with 3 or 4, segmented metapterygial axis with 10 to 12; metapterygial axis elongate, much larger than anterior basals, with a distal set of segments; radials mostly divided into three segments (proximal, intermediate, and distal), intermediates shorter than proximals or distals, which are equal in length.

Pelvic fins somewhat larger than anal but smaller than second dorsal in area; pelvics in some males relatively smaller than those of females; pelvics triangular, with anterior margins slightly convex to nearly straight, apices broadly rounded to subangular, posterior margins nearly straight, free rear tips acute (slightly attenuate in some specimens), inner margins straight; pelvic anterior margins 2.6 to 2.8, posterior margins 1.7 to 1.9, and inner margins 1.4 to 1.6 in comparable margins of pectorals.

Claspers and associated secondary sexual structures of males generally similar in basic plan to those described for *Galeorhinus galeus* (as "*Galeus vulgaris*") by Leigh-Sharpe (1921), but differing in several details; claspers long, more slender and more angular distally than those of *Galeorhinus* with bluntly pointed, flattened tips (Figure 5A); claspers of adult males extending well beyond free rear tips of pelvics; clasper groove roofed over and closed by its overlapping sides from apophysis to hypophysis; small pseudosiphon present mediodorsally, its pouch extending anteriorly on clasper. Unlike *Galeorhinus*, the pseudosiphon aperture is much less prominent and is located relatively farther from the clasper tip. Cover rhipidion very large (scarcely developed in *Galeorhinus*), formed as a rounded flap completely covering rhipidion; rhipidion evenly rounded (wedge-shaped in *Galeorhinus*); pseudopoda present, dorsolateral and opposite to the rhipidion edge (as in *Galeorhinus*); unlike *Galeorhinus*, the pseudopoda is partially covered by another flap, here termed the exorhipidion, which originates laterad to the pseudopoda and extends posteriorly to cover part of the rhipidion. Hypophysis opening at level of pseudopoda, cover rhipidion, and anterior third of rhipidion.

Clasper skeleton studied from radiographs of six males. Terminology is modified from Jungerson (1899) and White (1936, 1937). One basal cartilage connecting clasper cartilage to pelvic basipterygium; a small beta cartilage present at the junction of basal cartilage and clasper cartilage; details of terminal cartilages not clear, but at least two terminals, a dorsal and a ventral, are present; clasper cartilages heavily calcified in adult males.

First dorsal fin triangular with height much less than length from origin to free rear tip; origin ill-defined, grading into predorsal ridge; anterior margin slightly concave basally but convex towards fin apex, with a 45 degree slope relative to body axis; apex acutely rounded, posterior margin somewhat concave, free rear tip slender, elongate, acute; base much longer than fin height, inner margin about 60 to 70% of fin height; end of first dorsal base about over adpressed apex of pectoral; pectoral free rear tip anterior to pelvic origins by a distance nearly or quite equal to lengths of pelvic bases.

Second dorsal fin generally similar in shape to first dorsal; its height about half length from origin to free rear tip; fin base about 1.4 to 1.5 times height; inner margin about 0.5 to 0.7 of height; origin of second dorsal posterior to mid-point between anal origin and posterior end of pelvic base; free rear tip of second dorsal opposite or slightly posterior to that of anal; second dorsal over twice area of anal.

Anal fin a low triangle, with height about 0.4 in length, anterior margin broadly convex, apex rounded, posterior margin moderately concave, free rear tip slender and acute, and inner margin concave; inner margin almost or quite equal in length to height; fin base 1.4 to 1.6 times fin height.

Dorsal margin of caudal nearly straight, pre-ventral margin broadly convex, and junction of pre-ventral and post-ventral margins rounded; post-ventral margin long, concave anteriorly but nearly straight posteriorly and curving abruptly upward into subterminal notch; subterminal margin nearly straight, terminal margin invariably frayed but apparently moderately concave.

Vertebral counts given in Table 3.

Vertebral calcification pattern was studied from transverse sections and radiographs of centra from below first dorsal fin. Terminology for vertebral parts follows Ridewood (1921). Primary double cone without diagonal calcified lamellae; solid dorsal, lateral, and ventral inter-medialia present, separated by uncalcified areas for the basidorsals and basiventrals (Figure 3C); notochordal canal at constricted portion of double cone unusually large (as in many other

deepwater sharks, a feature possibly correlated with habitat).

The chondrocranium was dissected out in one specimen but is not described here. It is similar in structure to the crania of *Galeorhinus* and *Mustelus* described by Gegenbaur (1872) but differs in numerous details from both.

Stomach very large, subdivided into a sack-like fundus and a long slender pylorus. The fundus extends posteriorly over two-thirds the length of pleuroperitoneal cavity, then reverses direction as the pylorus to continue anteriorly nearly to root of liver, where it joins the spiral intestine. The latter is fusiform, with a spiral valve of about five turns (Figure 3D). The narrow rectum has a slender rectal gland attached distally to the epigonal organ in both sexes. Liver only moderately large, with paired lateral lobes concealing small medial lobe, posterior ends of lateral lobes extending only one-half to two-thirds of distance to posterior end of pleuro-peritoneal cavity. Spleen elongate, not nodular, originating dorsally on distal end of fundus and coursing anteroventrally on pylorus to spiral intestine, where it extends posteroventrally to below the first intestinal valve. Pancreas elongate,

TABLE 3.—Vertebral numbers in male and female *Iago omanensis*.

Monospondylous precaudals	Diplospondylous precaudals	Caudal vertebrae	Total vertebrae
Males			
34	48	48	130
34	47	48	129
36	49	58	143
36	47	52	135
37	49	51	137
37	45	51	133
37	51	44	132
Females			
37	51	50	138
37	47	50	134
37	51	58	146
37	51	52	140
37	51	50	138
38	51	51	140
38	51	51	140
39	52	53	144
42	49	56	147

single, located anterior to spiral intestine and dorsal to stomach. Ovaries well-developed only on right side, with long epigonal organ extending posteriorly to rectal gland; both oviducts well-de-

veloped and functional in all adult females examined, with small nidamental glands almost obsolete on the right side in some specimens; both testes apparently functional, subequally developed in three males examined, with a single epigonal organ attached to left testes. Semilunar valves of conus arteriosus in two rows, the anterior one with three valves, the posterior with three much smaller valves located each on the posterior base of an anterior valve.

Color brownish or grayish above and lighter below, with no conspicuous markings or abrupt color changes from dorsal to ventral; expanded chromatophores in the darkest specimen give a peppered appearance; small areas of darker pigmentation present near tips of both dorsal and caudal fins and in some specimens extending along leading edges of fins; lining of buccal cavity and peritoneum whitish.

VARIATION

The variation in morphometrics among our 16 specimens is substantial, unusually so for a series of adult sharks. Most of the differences do not follow sex, but it is apparent that the abdominal section is longer in females than in males. Thus the distance between pectoral and pelvic bases ranges from 13.7 to 16.1% of total length in seven males but is 16.8 to 20.0% in nine females. This is similar to the situation reported for the squaloid *Euprotomicrus bispinatus* by Hubbs, Iwai, and Matsubara (1967) and in *Carcharhinus leucas* by Thorson, Watson, and Cowan (1966). Large variations in tooth row and vertebral counts were noted also. Despite the range of variation between individuals and the sexual dimorphism in our sample, we find nothing to indicate that more than one species is represented or that the variation can be attributed to known geographical or environmental influences.

REPRODUCTION

One 440-mm specimen in our series has partially developed and uncalcified claspers but has eggs with very early embryos in the oviducts.

Thus the specimen is, at least functionally, a female. Histological examination of the ovaries was not made, but gross examination revealed one ripe ovary of normal appearance but little development of the other gonad. A similar instance of the partial development of claspers by a functional female *Centrophorus lusitanicus* was reported by Cadenat (1960). A more extreme example, recorded by King (1966), was of a hermaphroditic *Scyliorhinus caniculus* with a single immature clasper, a ripe ovotestis (with ovarian follicles at all stages and seminiferous tubules with mature sperm), and functional nidamental glands, oviducts, vasa deferentia, and seminal vesicles (with sperm). King also listed another *S. caniculus* specimen with two immature claspers, a ripe ovotestis, and oviducts, but no seminal vesicles and vasa deferentia. The opposite condition was found in a field-dissected specimen of *Mustelus higmani* by Dr. John Thompson (Springer and Lowe, 1963). This individual lacked claspers but had a pair of enlarged testes.

It may be significant that in the above cases the size of each shark was within the range of its functional sex at maturity regardless of external characters belonging to the opposite sex. The *Iago* and *Centrophorus* females with claspers were larger than would be expected for mature males of the species, but the clasperless male *Mustelus* was smaller than mature females of its species. Both hermaphroditic *Scyliorhinus* were the size of adult females of their species despite the presence of claspers.

Our smallest male, 224 mm long, is immature with uncalcified claspers but six others from 295 to 363 mm are mature. We did not examine internally a 358-mm female, the smallest of its series, but eight others from 395 to 582 mm are mature and have eggs in their oviducts. The eggs are for the most part not large, having yolks not more than 10 mm in diameter, and in our specimens, embryos, when present, are in a very early stage of development. In the oviducts each egg is encased in a thin and soft membranous shell which closely adheres to the oviduct lining. The nidamental glands vary in size from scarcely visible enlargements of the anterior oviduct to about 10 mm in diameter,

but all are far smaller than those present in oviparous scyliorhinids. The condition of nidamental glands and eggshells indicate that *Iago omanensis* is livebearing, with oviductal egg counts suggesting a litter of 2 to 10 young. The relatively small size of egg yolks implies that a maternal source of nourishment is provided the embryos unless the young are extremely small at birth.

SIZE

Iago is one of the smaller carcharhinids. In the Carcharhinidae, *Scoliodon*, the *Protozygaena* group in *Rhiodoprionodon*, and *Mustelus* have species nearly or quite as small as *I. omanensis*, though *Eridacnis* species are even smaller. One of the latter, *E. radcliffei*, is apparently the smallest carcharhinid and one of the smallest sharks, with males mature at 186 mm and females at 216 mm.

Size disparity between the sexes is a common phenomenon among elasmobranchs, in all known cases with females larger than males. In *Iago omanensis* this disparity is very marked; our largest male (365 mm) was only 63% as long as the corresponding female (582 mm) and weighed but one-sixth as much.

FOOD

Stomachs of two specimens contained remains of unidentified fish, in one a fish head 32 mm long and in the other a 50-mm section of the posterior trunk of a fish estimated to have been more than 200 mm long.

DISTRIBUTION

Table 1 shows the distribution of 16 of 17 known specimens of *Iago omanensis*, all except the holotype from IIOE Cruise 4B. Only three other shark specimens, all *Mustelus* sp., were collected during Cruise 4B from 81 trawling stations in the northern Arabian Sea. This total of only 19 shark specimens of two species is much lower than the expected catch for comparable gear in many other areas of continental shelf and slope.

A possible explanation for the low incidence of sharks in the catches lies in frequent presence

of poorly oxygenated water near the bottom along the coast between the Gulf of Kutch and the Gulf of Oman (See Banse, 1968, for a general account of the hydrography of part of this area). Sharks of species commonly held in marine aquaria are thought to require a high dissolved oxygen level for survival although studies to verify this for particular species have not been made.

Low oxygen concentration in water at the bottom, 0.22 to 0.77 ml/liter, is associated with five of the six IIOE stations at which *Iago omanensis* was taken. It appears that this species may be exceptionally tolerant to low oxygen levels, even at the moderately warm (16.24° to 22.39° C, or about 61.3° to 72.4° F) water it apparently inhabits. In the Red Sea, Marshall and Bourne (1964) reported that their unidentified carcharhinoid (which may be *Iago omanensis* or a close relative) occurred at depths down to 2195 m. As this area and these depths may have oxygen concentrations lower than 1 ml/liter at the end of summer (Richards, 1957), the Marshall and Bourne shark may be able to survive oxygen levels as low as known *Iago omanensis* apparently does in the Arabian Sea.

Gibbs and Hurwitz (1967) regarded the greater development of gill lamellae in the stomiatoid fish, *Chauliodus pammelas* compared with that in *C. sloani* as an adaptation to the low oxygen habitat of *C. pammelas*. We looked at structures having respiratory functions in *Iago omanensis* but found nothing to suggest such an adaptation. *I. omanensis*, however, has no closely allied species as a basis for comparison.

ACKNOWLEDGMENTS

We thank Dr. N. B. Marshall, British Museum (Natural History) for assistance in comparisons of the holotype with specimens of the IIOE series, Maarten Korrynga, Stanford University, for suggesting the generic name, and Mrs. Martha J. Mitchill of Kent Cambridge Scientific Inc., and Stanford University, for permitting one of the writers (Compagno) to use a Cambridge Stereoscan scanning electron microscope in her care.

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UPTAKE, ASSIMILATION, AND LOSS OF DDT RESIDUES BY

Euphausia pacifica, A EUPHAUSIID SHRIMP

ABSTRACT

JAMES L. COX¹

Euphausia pacifica Hensen, an abundant euphausiid shrimp from the California Current, can acquire sufficient DDT residue from its food to account for amounts found in its tissues. Assimilation efficiencies for DDT in ingested food are similar to published figures for assimilation of carbon from food. The concentration vs. size function suggested by gas-liquid chromatographic analyses of DDT residues in *E. pacifica*, however, was quite different from the function predicted by a theoretical food assimilation model. Direct uptake of ¹⁴C-DDT from water was rapid and partially reversible by returning animals to unlabelled flowing seawater. Uptake equilibrium was reached within 72 hr for smaller animals (<3 mg dry weight); larger animals apparently equilibrated after a longer period. ¹⁴C-DDT present in animals after 2 weeks exposure to unlabelled flowing water was retained in higher amounts in larger animals (>3 mg dry weight). The possible effects of dietary changes, moulting, and surface to volume ratios on observed natural levels are discussed.

DDT and its congeners are manmade substances which have achieved global distribution. This fact has produced widespread concern over their long-term impact in ecosystems and has stimulated efforts to study DDT transport from a systems analysis viewpoint (Harrison et al., 1970). Indirect evidence (Cox, 1970) suggests an accretion of DDT residues in oceanic food chains and underscores the need to produce information about mechanisms and rates of DDT acquisition and loss by plankton organisms. This paper reports the results of an experimental study of the euphausiid crustacean *Euphausia pacifica* dealing with quantitative aspects of DDT acquisition from food and water, rates of loss of acquisition from food and water, rates of loss of acquired DDT, and factors affecting equilibration with the surrounding water.

Euphausiid crustaceans are among the most abundant zooplankters in many oceanic regions. They are the food of commercially important fishes and in general represent an important link of oceanic food chains. *E. pacifica* is the most abundant euphausiid of the California Current. Ponomareva (1954, 1955, 1959, 1963) has sum-

marized behavioral and population data on this species, and Lasker (1966) has made extensive laboratory studies of its feeding, growth, respiration, and carbon utilization.

METHODS AND MATERIALS

Laboratory maintenance of *E. pacifica* has been described by Lasker and Theilacker (1965). Animals were maintained in a 40-liter capacity tub with flowing seawater at 10 to 12° C and fed daily rations of freshly hatched *Artemia* nauplii. Individuals were kept long enough during the course of the experimental work for noticeable growth. Mortality was extremely low after the first day that the animals were kept in the tub.

In direct uptake experiments, ¹⁴C-DDT was added in small carrier volumes of ethanol (ca. 100 μ liter) to GFC glass fiber filtered seawater (volumes from 1 to 10 liter) under constant stirring from a magnetic stirrer. Animals were introduced in groups from a small net or turkey baster. At the completion of an uptake run, animals were removed, rinsed briefly with fresh water, and placed in a desiccator for 6 days at

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room temperature. Losses of ^{14}C -DDT during desiccation were insignificant. Dried animals were removed, weighed on a Cahn electrobalance to ± 0.01 mg, placed in scintillation vials with equal volumes of NCS solubilizer (Nuclear-Chicago), and digested 1 hr at 70°C before introduction of scintillation fluid and subsequent counting on a Nuclear Chicago Unilux II scintillation counter.²

Loss experiments were done by taking labelled animals, subsampling them for initial ^{14}C -DDT levels, and placing them back in a flowing seawater tank. Water in the tank had a turnover time of less than 10 min, so lost ^{14}C -DDT was rapidly removed from the system. Groups of animals were removed from the tank at intervals and analyzed as described above.

In addition to work with ^{14}C -DDT, freshly caught *E. pacifica* were processed and analyzed for naturally occurring levels of DDT residues according to published methods (Cox, 1970), except that whole euphausiids were ground in the homogenizer, rather than algae on filters.

All direct uptake work was done at concentrations less than 33 ppt (parts per 10^{12}) ^{14}C -DDT in seawater, ranging down to 5 ppt. In uptake and loss experiments, individual samples were taken by removing about 10 to 15 animals from the experimental system, processing them, and plotting the results on log-log (full logarithmic) paper and fitting a least-squares regression line to the logarithmically transformed data. Depending upon the extent of the dry weights of the animals taken in each of the described groups, points corresponding to 1.0, 2.0, 3.0, or 10.0 mg dry weight were taken from the regression line for comparisons.

RESULTS

UPTAKE

Since the lipid constituents of planktonic organisms are not in direct contact with seawater, it is necessary to postulate a two-step process of uptake of DDT residues—first, adsorption on

to surfaces in contact with seawater and second, diffusion or transport of the adsorbed residues into the lipid constituents of the organism. Initial uptake by *E. pacifica* was rapid; Figure 1 shows the results of a 2-hr uptake experiment. Approximately equal numbers of animals were added to two 7-liter jars containing ^{14}C -DDT at a low ppt concentration. Two hours later, animals were removed and analyzed. The concentration vs. dry weight functions were found to be exponentials, yielding a straight line on the

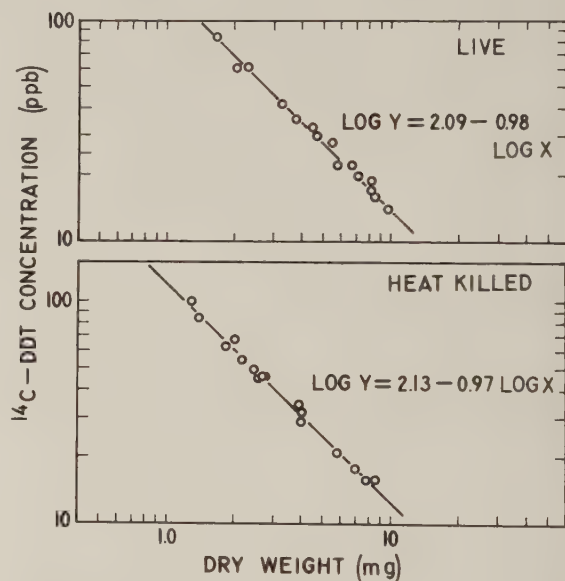


FIGURE 1.—Uptake of ^{14}C -DDT by *Euphausia pacifica* of different weights after 2 hr of exposure to labelled medium.

log-log plot. Initial uptake appeared to be unrelated to the animals' activity or respiration since heat-killed animals had the same total uptake as live animals. The amounts of ^{14}C -DDT taken up per animal were almost identical in these experiments (exactly equal amounts would yield a slope of -1 in the regression function).

In a different series of experiments, the slopes of the log-log concentration vs. dry weight functions changed from -1.05 at 2 hr and -0.99 at 8 hr to -0.67 at 24 hr. This change resulted from increased uptake by larger animals after longer exposure. Figure 2 summarizes the over-

² Reference to trade name in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

all patterns of uptake for the 72-hr period. Three arbitrary dry weights of animals (2.0, 3.0, and 10.0 mg) were chosen to illustrate different weight effects during uptake. The points corresponding to these dry weights were taken from regression lines like those shown in Figure 1. The values on the ordinate were converted from concentration to total picograms ($g \times 10^{-12}$) of ^{14}C -DDT. After 72 hr of exposure, the 10-mg animal did not reach equilibrium; the 2 and 3 mg animals did reach equilibrium after 72 hr.

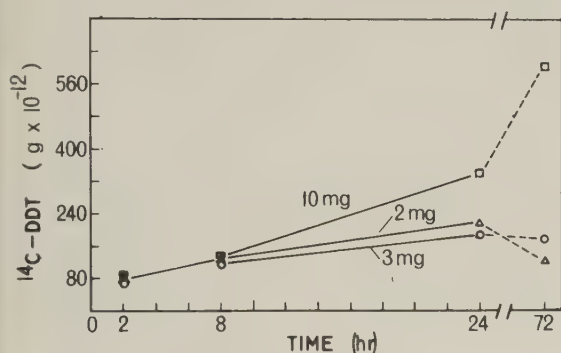


FIGURE 2.—Uptake of ^{14}C -DDT by *Euphausia pacifica* in a closed system. Equilibrium concentration of ^{14}C -DDT in the water was 20 parts per trillion. The three dry weight values were taken from log-log regression lines for subsamples of 10 animals or more. See text for details.

EFFECT OF TEMPERATURE

Temperature appeared to have little effect on initial uptake rates. The Q_{10} for short-term (2 hr of exposure) uptake between 5° and 15°C for an animal of a given dry weight was computed by comparing log-log regression functions for two groups of animals exposed to the same nominal concentrations of ^{14}C -DDT in the medium—one group at 5°C and the other at 15°C . This procedure yielded a Q_{10} of 1.11 for an animal of 2.0 mg dry weight and a Q_{10} of 1.29 for an animal of 10.0 mg dry weight. Both figures suggest a physical process as the limiting step for direct uptake of DDT; the higher figure for

larger animals may reflect a higher Q_{10} for transfer into the lipid reservoir of the larger animal.

Del Nimmo (personal communication, 1970) has evidence that DDT residues are transported to internal sites of accumulation by a protein fraction in the haemolymph of penaeid shrimps. If *E. pacifica* is comparable in this regard to the penaeid shrimp, then transport of DDT in the circulatory system must not be the rate-limiting step in uptake, since circulatory rates may be expected to have a higher Q_{10} than those found. Respiratory rates, which are directly dependent upon circulatory rates, exhibit Q_{10} values in excess of 2.2 in *E. pacifica* (Paranjape, 1967).

CONCENTRATION FACTORS

The short-term uptake concentration factors (the ratio of the concentration of DDT in the animals to the concentration in the water after brief exposure) for ^{14}C -DDT changed little over the range of concentrations employed. Table 1 summarizes data that were taken from log-log plots for animals of 1.0 and 3.0 mg dry weight. It is evident that short-term uptake of DDT for an animal of a given size is proportional to the concentration of the DDT in water.

TABLE 1.—Concentration factors after 2 hr exposure.

Equilibrium concentration of ^{14}C -DDT in seawater	Concentration factor $\times 10^3$ Concentration in animal (dry): concentration in water (w/v)	
Parts per trillion	1.0 mg	3.0 mg
5	4.4	1.1
20	3.2	1.1
26	4.1	1.2
33	4.1	1.2

¹ This includes data from one-half hour run.

LOSS

If short-term exposure to DDT in the seawater medium of *E. pacifica* results in surface adsorption, one expects that these adsorbed residues will be lost to the medium if the ambient concentration of the DDT is lowered. If all the labelled DDT in a short-term experimental exposure is adsorbed, the animals would be expected to lose eventually all of their label when returned to unlabelled flowing seawater.

Figure 3 shows the results of 2 weeks of "rinsing" on animals originally exposed to ^{14}C -DDT for 2 hr. The lower data points show that a fraction of the ^{14}C activity was retained, although the size vs. estimated ^{14}C -DDT concentration function was altered considerably by the treatment. Figure 4 shows a loss curve constructed from a series of log-log plots such as those in Figures 1 and 3. The 10.0 mg animal apparently neared equilibrium at the end of the 2-week period, but the 2.0 and 3.0 mg animals were still declining. Presumably, the ^{14}C -activity loss occurred by diffusion of the parent compound (^{14}C -DDT) or metabolites into the flowing seawater medium. Some loss may have occurred through moulting. Unfortunately, the conditions of the experiment did not allow any record of moult production.

ASSIMILATION FROM FOOD

Animals were isolated in Carolina dishes and kept at 10°C in the dark in 200 ml of GFC filtered seawater and fed known numbers of freshly hatched *Artemia* nauplii previously labelled with ^{14}C -DDT ($2.7 \pm 0.02 \times 10^{-12}$ g ^{14}C -DDT/nauplius, on the average for groups of 10 to 50). After 24 hr, animals were removed to new dishes and fed daily rations of unlabelled nauplii to ensure flushing of the undigested remains of the labelled nauplii from the guts of the experimental animals. After 2 days, the animals were removed, rinsed, dried in a desiccator, and weighed. Amounts of ^{14}C -DDT activity retained were computed by measuring the activity of the dried animals as described in the section on methods. Amounts of labelled nauplii eaten were calculated by counting the numbers left in the dishes after the 24-hr feeding period. Table 2 summarizes the results of the experiment.

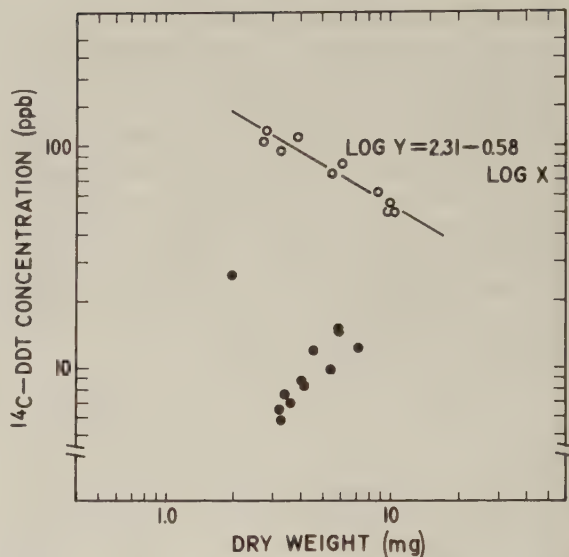


FIGURE 3.—Loss of ^{14}C -DDT from *Euphausia pacifica* kept in a flowing water system. Values for the different dry weights were obtained as indicated in the methods section of the text. The solid dots indicate ^{14}C -DDT concentrations after 2 weeks of exposure to unlabelled flowing seawater. The open dots are for animals exposed to ^{14}C -DDT for 2 hr, then "rinsed" in the flowing seawater system for 2 hr before sampling.

Animal 5 may have had a higher assimilation efficiency because of delayed excretion of the gut contents, presumably attributable to the post-moult condition, i.e., passivity and lack of feeding or swimming movements (Paranjape, 1967). Consequently animal 5 was excluded from further calculations. Animal 1 may have had a lower assimilation efficiency because some loss of labelled material with the moult. The average ^{14}C -DDT assimilation efficiencies for animals 2 to 4 is only slightly lower than Lasker's (1966) estimates of carbon incorporation efficiency for *E. pacifica*.

TABLE 2.— ^{14}C -DDT assimilation from labelled *Artemia* nauplii by *Euphausia pacifica*.

Animal	Nauplii eaten nauplii offered (labelled)	Percent consumption	Amt. ^{14}C -DDT ingested picograms ($\text{g} \times 10^{-12}$)	Amt. ^{14}C -DDT assimilated picograms ($\text{g} \times 10^{-12}$)	Moult ¹		Percent assimilation efficiency
					Pre	Post	
1	30/30	100	81	28	—	+	34
2	44/53	83	119	70	—	—	58
3	38/38	100	103	81	—	—	78
4	49/75	65	132	106	—	—	80
5	21/62	34	62	58	+	—	93

¹ Premoult means moult was recovered after feeding on labelled nauplii. Postmoult means moult was recovered after feeding on unlabelled nauplii.

In another experiment, 12 animals were placed in a vessel and fed ^{14}C -DDT labelled nauplii for 1 hr. Six animals were taken and processed for ^{14}C activity, and the remaining six were allowed to feed on unlabelled nauplii for 2 days before they were processed. The assimilation efficiencies were computed as a ratio of ^{14}C activity in the animals processed after 2 days to the ^{14}C activity in the animals processed immediately after the 1-hr feeding period. This method yielded an assimilation efficiency of 76%.

For calculations, I took a mean of the first four animals' assimilation efficiencies. It is uncertain whether this figure (62%) adequately reflects the influence of moulting on DDT assimilation efficiency. Moulting probably plays an

important role in DDT loss from the organism; DDT incorporated into the moult is lost when the moult is shed.

NATURAL LEVELS OF DDT

Figure 5 shows the results of gas chromatographic analyses of *E. pacifica* samples collected in August 1970, the same time that most of the experimental animals were collected. On the basis of DDT acquisition from food, a rising trend in the DDT residue concentrations would be expected as animals grew and aged. In order to examine the discrepancy between the observed DDT values and that which might be expected from cumulative assimilation of DDT residues from food, a model was constructed.

Woodwell, Wurster, and Isaacson (1967) found 0.04 ppm in plankton hauls from a polluted estuary; I have found 0.25 ppm in large, pooled samples of copepods from Monterey Bay. The mean weight of these copepods, 0.95 mg, was only slightly higher than for those eaten by *E. pacifica*. *E. pacifica* also feeds on phytoplankton. The concentrations of phytoplankton, when the density of the standing crop of phytoplankton is high enough to stimulate feeding, are probably below 0.1 ppm, wet weight (Cox, 1970). An intermediate figure can be taken as representative of the DDT concentration of the food of *E. pacifica*. I chose 0.1 ppm as the mean concentration of DDT residues in food.

Employing the carbon budget parameters published by Lasker (1966) and the estimate of DDT residue concentration in food organisms, I calculated the cumulative DDT content of the ingested food of animals of three different dry weights (Table 3). The computed values are compared with values interpolated from the arbitrarily drawn dotted line in Figure 5.

Two conclusions may be drawn from a comparison of columns 7 and 8 in Table 3. First, the estimated values are close enough to the observed values to indicate that ingestion is a sufficient source of DDT residues in *E. pacifica*. Second, the concentration vs. size function of the observed values is quite different from that of the calculated values, indicating that processes other than simple accumulation of a fraction of

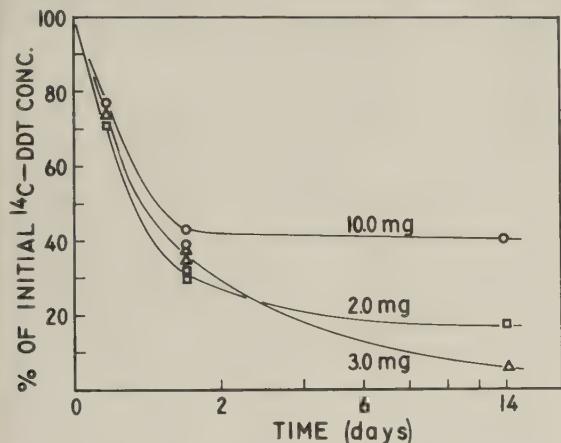


FIGURE 4.—Loss curve constructed from data such as that presented in Figure 3. See text for details.

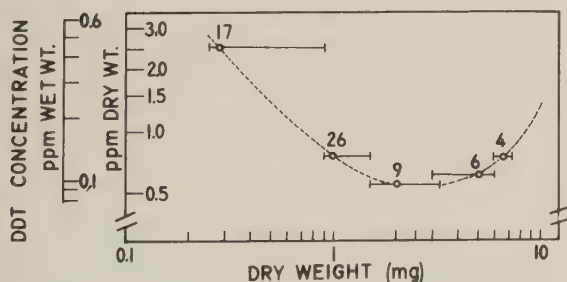


FIGURE 5.—DDT residue concentrations in different sizes of *Euphausia pacifica*. The numbers next to the data points indicate the numbers of animals in the pooled sample analyzed; horizontal brackets indicate the range of weights of individual animals within the groups.

TABLE 3.—Calculation of expected DDT residues in different sizes of *Euphausia pacifica*.

Dry weight	Equivalent weight carbon	Carbon growth incorporation efficiency ¹	Cumulative amount nauplius carbon required	DDT equivalent ² ($\mu\text{g} \times 10^{-9}$)	Assumed DDT incorporation efficiency	Parts per 10 ⁶ — dry	
						Expected DDT concentration	Observed DDT concentration
mg	mg		mg				
1.0	0.42	0.30	1.4	1.4	0.62	0.9	0.75
2.0	0.84	0.15	4.2	4.2	0.62	1.3	0.55
3.0	1.26	0.10	8.4	8.4	0.62	1.7	0.56

¹ Carbon growth incorporation efficiencies were taken from Table 2 of Lasker (1966); the figures shown are not means of the values presented by Lasker but are round-figure approximations which take account of the trends shown and of the different range of sizes of animals used in the laboratory experiments which yielded these figures.

² The DDT equivalent of the food was calculated from nauplius carbon assuming a wet weight DDT concentration in the food of 0.1 ppm, and a carbon weight to wet weight ratio of 0.1.

ingested DDT determine DDT residue concentrations in *E. pacifica*.

DISCUSSION

For *E. pacifica*, there are two important sources of DDT residues—direct uptake from water and assimilation from food. Short-term direct uptake is rapid and appears to be at least partially reversible, suggesting adsorption of DDT to exposed surfaces. Over longer periods, these initially acquired residues are transferred to internal deposition sites. The long-term uptake and loss experiments show that larger animals tend to retain more of the initially acquired DDT, possibly because of greater lipid content. Direct uptake from water is a possible mechanism for accumulation of residues if the initially adsorbed residues are continually transferred to internal deposition sites. The rate of initial uptake will depend upon the concentration in seawater (Table 1); retention of these initially acquired residues apparently depends on other factors, judging from the lower set of data points in Figure 3. One determinative factor may be lipid content; values given by Mauchline and Fisher (1969) indicate that lipids, expressed as percentage of body weight, can vary by as much as an order of magnitude in *Euphausia* spp., according to the body weight of the animal. The four lipid values listed by Mauchline and Fisher (1969) for *E. pacifica* correspond closely to the DDT concentration values after 2 weeks rinsing shown in Figure 3. However, in the absence of concurrent lipid values for the animals of the lower data points shown in Figure 3, no conclusion can be drawn about the relation-

ship between retention of ¹⁴C-DDT and the percentage lipid composition of the animals. It is reasonable to assume, nonetheless, that the changes in the lipid content of *E. pacifica* which accompany reproductive cycles and seasonal feeding changes will have some impact on the DDT residue content, regardless of the source of the DDT residues.

The second possible source of DDT residues, as previously discussed, is from food. In this case, DDT is almost certainly transported directly in the fat of the food organisms to the fat reservoir of the consumer. Numerous studies indicate that marine organisms do not alter lipids from ingested food (Lasker and Theilacker, 1962; Jezyk and Penicnak, 1966; Jeffries, 1970; and others). Comparison of published values of fatty acid composition for *E. pacifica* (Yamada, 1964) with values for its food, microzooplankton and phytoplankton (Jeffries, 1970), suggests that mass assimilation of fatty constituents along with DDT residues is taking place.

As has been suggested, food is probably a sufficient source of DDT residues in *E. pacifica* (Table 3). Direct uptake may contribute to DDT residues in *E. pacifica*, but its role cannot be assessed because of the lack of seasonal data on DDT concentrations in seawater as well as uncertainties about DDT's availability to organisms in the natural environment (Cox, 1971).

Some basis must be sought to explain the unexpected higher concentrations of DDT residues in the smaller animals. Three possibilities exist: (1) the food of immature *E. pacifica* may have higher DDT concentrations, (2) direct uptake from water is more important for the smaller animals because of their higher area:volume

ratios, or (3) smaller animals have not used any of their lipid reserves, which use may cause loss of some DDT residues. The data presented here do not allow conclusions on the relative importance of these possibilities.

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A KEY TO THE AMERICAN PACIFIC SHRIMPS OF THE GENUS *Trachypenaeus* (DECAPODA, PENAEIDAE), WITH THE DESCRIPTION OF A NEW SPECIES

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ABSTRACT

Study of American Pacific members of the genus *Trachypenaeus* reveals that variation in armature of the telson includes not only movable spines, but also fixed spines and even no spines at all. It also confirms that the eighth somite bears two arthrobranchiae instead of one arthrobranchia and one pleurobranchia. A new species, *Trachypenaeus fuscina*, is described, the specific features of *T. faoea* Loesch and Avila are presented, and a key to the five members of the genus occurring in the region, together with their ranges, is included.

Along the Pacific coast of Latin America species of *Penaeus* are the mainstay of the shrimp fisheries; however, members of various other genera contribute to the catches in significant quantities. Among the latter, three *Trachypenaeus* have been previously recognized: *T. byrdi* Burkenroad, *T. similis pacificus* Burkenroad, and *T. faoea* Loesch and Avila. A fourth, noncommercial species, *T. brevisuturæ* Burkenroad, is also found in the region. Burkenroad (1934a, 1938), presented detailed descriptions of the taxa he described, but the characters cited for *T. faoea*, except color pattern, have not proven to be diagnostic. Since the commercial *Trachypenaeus* are indiscriminately known by the common names of "tigre" and "cebra," a definition of *T. faoea* is needed.

The study of collections of American Pacific *Trachypenaeus* has shown that yet another commercial species of this genus occurs in the area. It also pointed out a previously undescribed variation in the armature of the telson, and confirmed the identity of the gills on the eighth somite.

The measurement of total length is the linear distance from tip of rostrum to posterior end of telson, and that of carapace length is the distance from orbital margin to midposterior margin of

carapace. The ratio, length of posteriormost pair of telsonic spines to width of terminal portion of telson, is presented in the following manner: length of spine/width of terminal portion = average ratio (*N*, number of specimens: range of variation).

GENUS *Trachypenaeus* ALCOCK

Trachypeneus Alcock, 1901: 15.—Burkenroad, 1934a: 49.—Burkenroad, 1934b: 73, 94.

Trachypenaeus.—Kubo, 1949: 391.—Dall, 1957: 202.

Type-species by original designation, *Penaeus anchoralis* Bate, 1881.

The telson of the genus *Trachypenaeus* was described by Kubo (1949) as lacking fixed spines, and by Dall (1957) as possessing several pairs of lateral movable spines. Previously, Burkenroad (1934b) had proposed a grouping of the genera of the subfamily Penaeinae in four series; he defined the series *Trachypenaeus* as having a variable number of mobile lateral spines on the telson and characterized the series *Parapenaeus* as possessing one to three pairs of movable spines in addition to a fixed posterior pair, considering the presence of fixed spines on the telson as a unique character for the latter series.

In the species of *Trachypenaeus* described below, however, the posteriormost of the four pairs of spines on the telson is fixed, and in *Trachy-*

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penaeus byrdi Burkenroad the telson lacks spines. The telson of the members of the genus, thus, must now be more broadly characterized as having several pairs of lateral movable spines, or several pairs of movable spines anteriorly and a fixed posterior pair, or unarmed. Furthermore, the evidence presented here indicates that the character of the posteriormost pair of telsonic spines is not a unique character of the series *Parapenaeus*.

The branchial formula of *Trachypenaeus* was given by Dall (1957) as follows: pleurobranchiae on somites IX to XII; a rudimentary arthrobranchia on somite VII, anterior and posterior arthrobranchiae on VIII to XII, and a posterior arthrobranchia only on XIII; mastigobranchiae (epipodites) on VII, VIII and XII [first, second maxillipeds and third pereopod], sometimes also on X and XI [first and second pereopods]. All American species possess this combination of branchiae, including epipodites on the first and second pereopods, and, in addition, a vestigial anterior arthrobranchia on somite XIII.

In *Trachypenaeus*, the anterior arthrobranchia on somite VIII is considerably displaced dorsally, and appears to occupy the position of a pleurobranchia; however, its attachment is on the arthrodial membrane. In Figure 1 the openings of the branchiae on somites VIII and IX, together with the proximal parts of the second and third maxillipeds, are depicted; this figure clearly shows that the two arthrobranchiae on somite VIII are attached to the arthrodial membrane, like those on somite IX, whereas the pleurobranchia on the latter somite has its origin on the pleural membrane.

Burkenroad (1934a; see also 1934b) divided the genus *Trachypenaeus* into two subgenera, *Trachypenaeus* and *Trachysalambria*, the latter possessing epipodites on the first and second pereopods, and a thelycum with a median pocket on sternite XIV; the former lacks these characters. Later, Burkenroad (1959) observed that some members of *Trachysalambria* lack such epipodites and, thus, questioned the "usefulness" of his division. Recently, a number of species from the Indo-Pacific have been described which bear epipodites on the first two pairs of pereopods but the thelyca, as indicated by Racek and

Dall (1965), differ from that Burkenroad attributed to the members of *Trachysalambria*. Consequently, more investigations are needed to interpret the interrelationships of the species of the genus. It should be pointed out, however, that all American species of *Trachypenaeus* exhibit the characters given by Burkenroad for *Trachysalambria*.

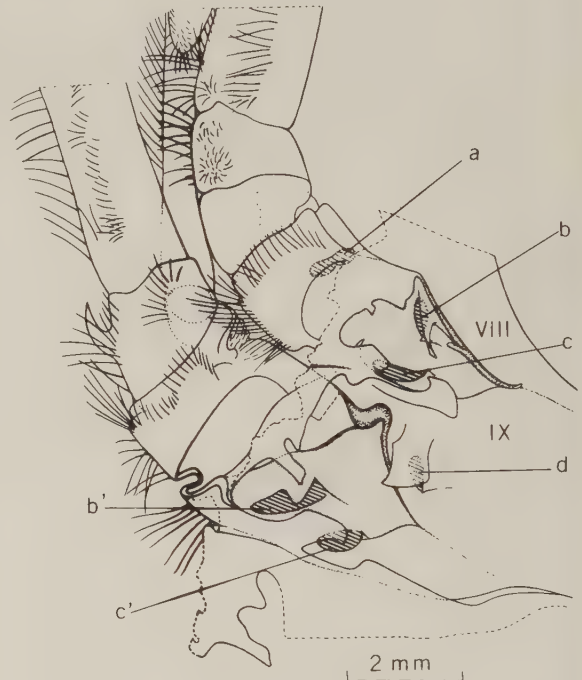


FIGURE 1.—*Trachypenaeus fuscina* sp. n., ♀ 33 mm carapace length, off Barra de San Marcos, Chiapas, México. Dorsal view of proximal part of second and third maxillipeds and attachments of gills on arthrodial and pleural membranes of somites VIII and IX (second maxilliped has been displaced laterally). a. Podobranchia. b, b'. Anterior arthrobranchiae. c, c'. Posterior arthrobranchiae. d. Pleurobranchia.

The spelling of the generic name used here, *Trachypenaeus* instead of *Trachypeneus* as was originally published, is based on the decision reached by the International Commission on Zoological Nomenclature, Opinion 864, 1969, Bull. Zool. Nomencl. Vol. 25, Parts 4-5, p. 138-147.

Trachypenaeus fuscina SPECIES NOVA

FIGURES 1, 2, 3A, 4A-F, 5A, 6

"PINTO," "CEBRA," "TIGRE,"

Trachypenaeus faoe Lindner, 1957 [part], *nomen nudum*: 48, 49, 81, 145.—Crocker, 1967 [part]: 8, 57.

MATERIAL

Holotype.—♀, USNM 135403, off Cocodrilo, Chiapas, México, 22 m, October 31, 1969, H. Romero and G. Gómez, 35.25 mm carapace length, 135 mm total length, ratio length of spine/width of terminal portion of telson = 0.55.

Allotype.—♂, USNM 135404, off La Tapada, Chiapas, México, 22 m, July 31, 1970, D. Palacios, 26 mm carapace length, 108 mm total length, ratio length of spine/width of terminal portion of telson = 0.80.

Paratypes.—México. Oaxaca. 8 ♀, IBUNM-USNM, Salina Cruz, May, 1961, E. Martín F. 1 ♂, INIBP, Santa María Xadani, Laguna Superior, July 23, 1970, I. Pérez Farfante. 1 ♀, USNM, off Las Chiches, 24 m, August 6, 1966,

Z. Ortiz and G. Gómez. 2 ♂ 1 ♀, USNM, Golfo de Tehuantepec, July 14, 1963, I. Mayés. Chiapas. 3 ♀, USNM, off Barra de San Marcos, 27 m, March 18, 1964, A. Guerra. 3 ♀, INIBP-USNM, off mouth of Río Suchiate, 7-13 m, February 12, 1968, Romero, Ortiz, Sánchez, and Arias. 1 ♀, USNM, off La Tapada, 7-9 m, February 5, 1968, Romero, Ortiz, Sánchez, and Arias. 4 ♂ 5 ♀, INIBP-USNM, off La Tapada, 22 m, July 31, 1970, D. Palacios. 5 ♀, INIBP-USNM, off Cocodrilo, 22 m, October 31, 1969, H. Romero, and G. Gómez. Ecuador. 1 ♀, USNM, off Playas, September 2, 1962, fishermen. Perú. 2 ♀, USNM, Caleta La Cruz, Tumbes, 70 m, E. M. del Solar.

DESCRIPTION

Carapace pubescent (Figure 2); dorsum densely covered by setae; paired bands of longer setae flanking postrostral carina, from rostrum to various levels in posterior third of carapace; another band on dorsal side of antennal carina; longer setae also along cervical, hepatic and branchiocardiac sulci, and others forming patches on orbital region and posteroventral portion



FIGURE 2.—*Trachypenaeus fuscina* sp. n. Lateral view, ♀ 33 mm carapace length, off Cocodrilo, Chiapas, México.

of antennal carina; branchial region covered with short setae; pair of small, bare, crescent-shaped to semicircular areas at anterior end of posterior third of carapace, flanking postrostral carina. Abdomen naked, except for elongate patches of long setae on each side of mid-dorsal carina on third to sixth somites, and two additional paired patches often present on sixth somite, one dorsal and other ventral to cicatrices. Telson (Figure 3A) with two pairs of longitudinal bands of long setae, one along walls of median sulcus, and other along lateral sulci.

Rostral teeth 6-7, first tooth situated immediately behind orbital margin; epigastric tooth at posterior end of anterior fourth of carapace. Rostrum reaching as far as proximal fourth of dorsal flagellum; basal portion ascending well above level of carapace, and apical one-third, unarmed portion, decreasing progressively in height, and curving upward. Adrostral carina slightly sigmoidal, ending about midway between first rostral and epigastric tooth. Postrostral carina strong, long, reaching almost to posterior margin of carapace, higher anteriorly, bearing elongate fossette, immediately behind midlength, and several pits posteriorly. Orbital angle produced into rather broad orbital spine. Gastro-orbital carina and orbito-antennal sulcus absent. Postocular sulcus deep, extending posteroventrally to about level of orbital angle. Longitudinal suture well marked, long, extending along two-thirds of carapace or slightly more. Transverse suture short, clearly distinct, situated at level of coxa of third pereopod. Antennal and hepatic spines long and strongly acuminate. Antennal carina prominent, extending to below hepatic spine. Cervical sulcus shallow and short, not intercepting longitudinal suture. Hepatic carina and hepatic sulcus well marked, and inclined anteroventrally, their length about one-third that of carapace. Postcervical line sinuous, extending from postrostral carina to near posterior end of hepatic carina. Branchiocardiac sulcus feeble, marked ventrally by obtuse carina. Pterygostomian angle obtuse, its ventral margin sloping posteroventrally before turning backwards.

Antennular flagella subequal, shorter than either antennular peduncle or carapace, slightly longer in males than in females of same length,

and proportionally longest in subadult; ratio of flagellar length to carapace length about 0.66 in shrimp of 15 mm carapace length, ratio decreasing with increasing length of shrimp to about 0.40 in shrimp with carapace length of 40 mm. First segment of antennular peduncle with disto-median border produced into heavy, scalelike projection densely covered with long setae; distolateral spine prominent, slender and sharp; prosartema long, extending to distal end of segment; stylocerite attaining midlength of segment.

Antennal flagellum long, almost twice total length of shrimp; scaphocerite reaching distal end of antennular peduncle, elongate, its length $2\frac{1}{2}$ times maximum width; lateral, thickened margin ending anteriorly in strongly pointed spine.

Third maxilliped surpassing carpocerite by as much as dactyl and one-fourth of propodus; first pereopod reaching, at most, base of carpocerite; second pereopod surpassing distal end of carpocerite by as much as three-quarters of dactyl; third pereopod exceeding carpocerite by as much as propodus and one-tenth of carpus; fourth pereopod extending to about same level as first; fifth pereopod very long and slender, exceeding carpocerite by entire length of dactyl, and surpassing fourth by propodus and four-fifths length of carpus. Spine on basis of third maxilliped, and, as in all members of genus, on first and second pereopods. Epipodites on first and second pereopods deeply bifurcate, epipodites on second maxilliped and third pereopod unfurcate; vestigial anterior arthrobranchia on somite XIII.

Abdomen with middorsal carina extending from posterior half of second to sixth somite, carina low and rounded on second, rather acute on third, and forming high and sharp keel from fourth to sixth somites; fourth and fifth somites with posteromedian V-shaped notch; sixth somite bearing middorsal spine posteriorly, small spine at posteroventral angles, and two cicatrices on each side, anterior one sensibly longer. Telson (Figure 3A) shorter than inner ramus of uropod, with median sulcus deep anteriorly and well marked posteriorly to base of terminal portion; paired rounded carinae flanking median sulcus, and sharp carinae bordering oblique, lateral

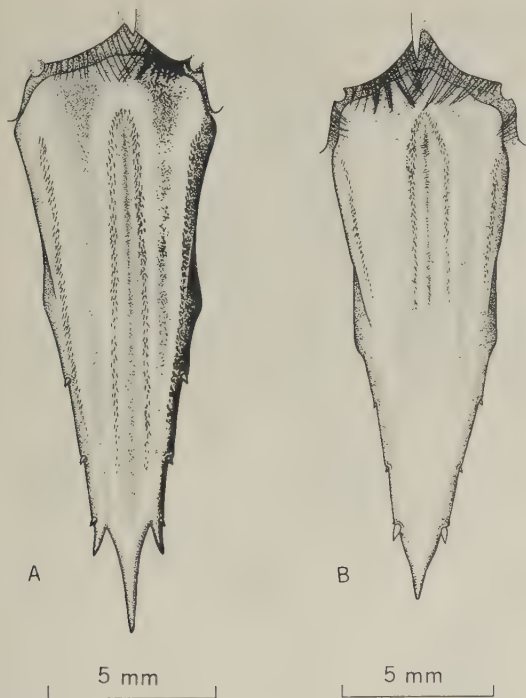


FIGURE 3.—Telsons. A. *Trachypenaeus fuscina* sp. n., ♀ 33 mm carapace length, off Cocodrilo, Chiapas, México. B. *Trachypenaeus faoea* Loesch and Avila, ♀ 37 mm carapace length, Ensenada de Garachiné, Golfo de Panamá, Panamá.

sulci; terminal portion (from mesial base of posteriormost spine to apex) from relatively short to long, its length relative to its width at base ranging from 1.60 to 3.70; telson armed with four pairs of lateral spines, posteriormost pair fixed and long, ratio length of spine/width of terminal portion = 0.70 (N 27: 1-0.50); other spines movable and strong, posterior pair (located at lateral base of longer fixed spines) relatively long, anterior two pairs (located posterior to midshoulder of telson) small, but usually visible with naked eye.

Petasma (Figure 4A, B) with large, hornlike, distolateral projections, broad at base, curving laterally, and opening dorsally near anterior margin by long, transverse slit; posteroventral wall of horns reflexed dorsally and produced forward into membranous flap; distomedian projec-

tions short, overhanging distoventral aperture of petasma. Ventrolateral lobule of petasma almost entirely cornified, including ventral wall of horns and dorsally reflexed lateral margin; proximomedian portion of lobule with rather flexible strip, tapering to base of distal two-fifths of median margin, there ending where corneous sclerite, curving mesially, reaches margin; soft elliptical area immediately distal to corneous section of margin. Dorsolateral lobule bearing narrow rib along proximomesial third, rib broadening proximally and turning mesially at proximal extremity resembling golf club. Dorsomedian lobule with narrow, distally bifurcate rib at base of distomedian projection, its length about one-third that of lobule. Length of petasma—from apex of distomedian projection to proximal margin of dorsolateral lobule—almost one and one-fifth times its width at level of distolateral projections.

In males, posterior margin of sternite XIII (Figure 4F) bearing large, elongate, subelliptical to ovate median plate, latter with obtuse to acute tip, and numerous marginal setae; anterior half of sternite XIV with strong, subpyramidal prominence, its anterolateral, ventromedially inclined edges often forming shelflike ridges.

Appendix masculina (Figure 4C-E) thick, subcircular in outline, its length along midline subequal to maximum width, and produced into two proximolateral prominences; dorsal wall cornified, except for distal and lateral margins, these, together with ventral wall, rather soft; entire, broad, median border setiferous, setae continuing on distal margin forming narrow band. Dorsal base of endopod with corneous, roughly trapezoidal sclerite, bearing strong median rib articulating distally with appendix masculina; rib with small setiferous depression on distal portion of ventral surface. Accessory sclerite on dorsolateral margin of endopod.

Thelycum (Figure 5A) with posterior part bearing anterolateral, subtriangular, heavily sclerotized projections; anterior part of sternite XIV, with platelike base, projecting as median prominence, and produced forward into pair of lateral, elongate, tongue-like flaps, extending al-

most to anterior margin of median plate; bases of flaps strongly inclined dorsomedially, forming depression limited posteriorly by prominence; margins of flaps often reflexed ventrally.

Platelike portion of sternite XIV naked; its lateral margins pronouncedly curving posteromedially, forming deep emarginations with projections from posterior part; basal portions of

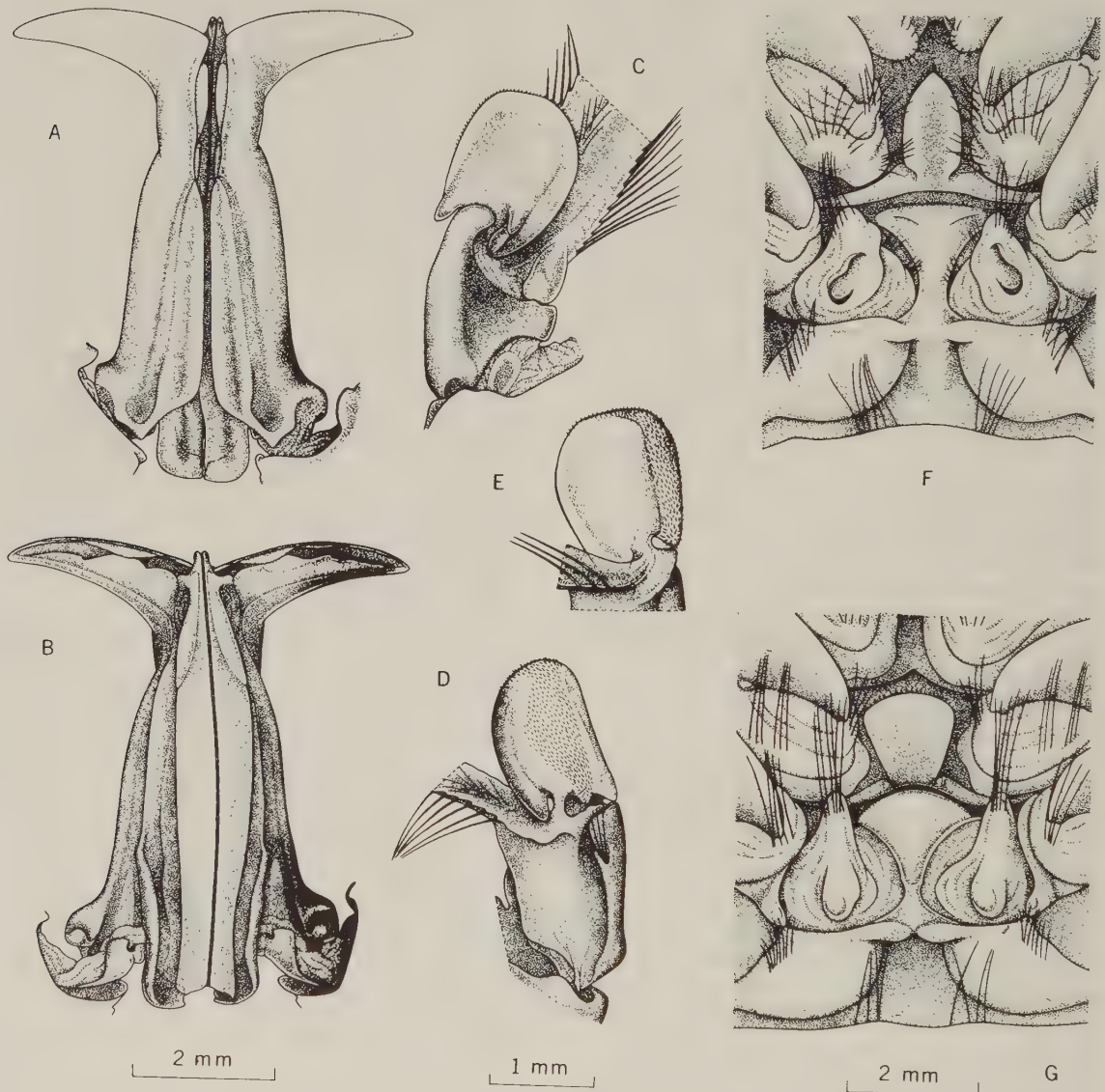


FIGURE 4.—*Trachypenaeus fuscina* sp. n., ♂ 20 mm carapace length, off Cocodrilo, Chiapas, México. Petasma: A. Ventral view. B. Dorsal view. Appendix masculina and proximal portion of endopod: C. Dorsal view. D. Ventral view (endopod displaced). E. Ventromedial view of appendix masculina. F. Sternites XIII and XIV. G. *Trachypenaeus faoea* Loesch and Avila, ♂ 18 mm carapace length, Playa Bella Vista, Panamá, sternites XIII and XIV.

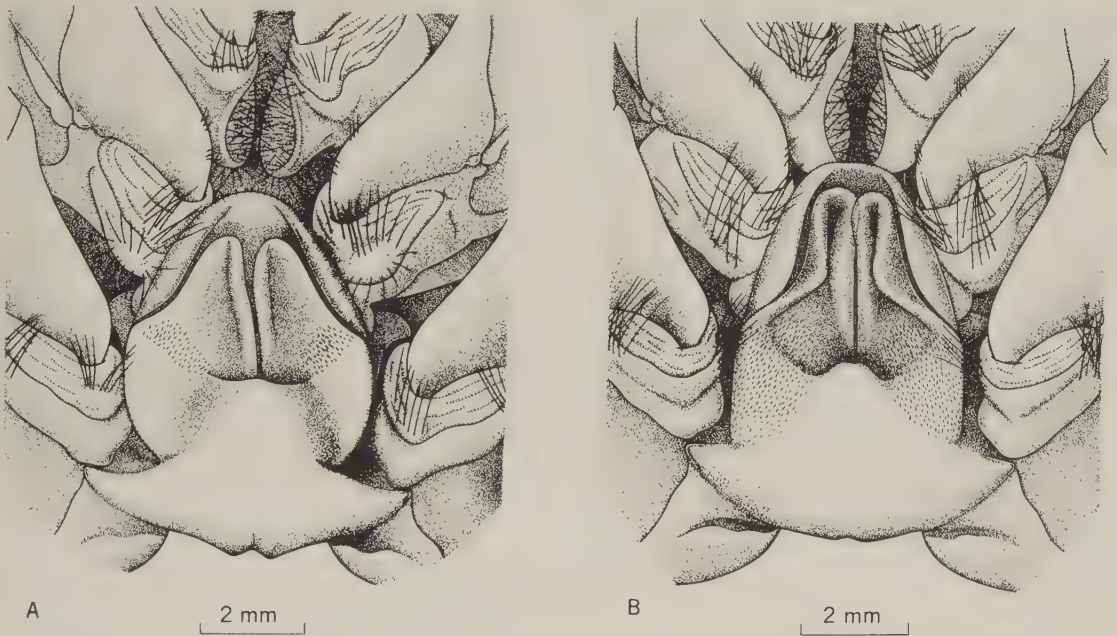


FIGURE 5.—Thelyca. A. *Trachypenaeus fuscina* sp. n., ♀ 34.5 mm carapace length, off Barra de San Marcos, Chiapas, México. B. *Trachypenaeus faoea* Loesch and Avila, ♀ 32.5 mm carapace length, Ensenada de Garachiné, Golfo de Panamá, Panamá.

flaps setose. Median plate of sternite XIII large, oval, strongly concave ventrally, with margin tumid, and bearing setae; plate extending posteriorly joining dorsal wall of flaps, thus giving rise to median pocket; latter bearing paired apertures of internal seminal receptacles laterally (Figure 6B). In impregnated females, median pocket occupied by sclerotized, sperm-free, brownish component of spermatophores, extending almost to anterior margin of median plate. Hardened, glutinous material, extruded with spermatophores, protruding through slit between flaps, forming plug on ventral surface of flaps.

Internal seminal receptacles (Figure 6A, B) consisting of paired, longitudinally arranged, trilobed, membranous sacs: single, large, posterior lobe, dorsal to median pocket, extending caudally almost to posterior margin of sternite XIV; and two small lobes, one directed antero-medially, dorsal to median plate, and the other laterally, dorsal to small hood of sternite XIV.

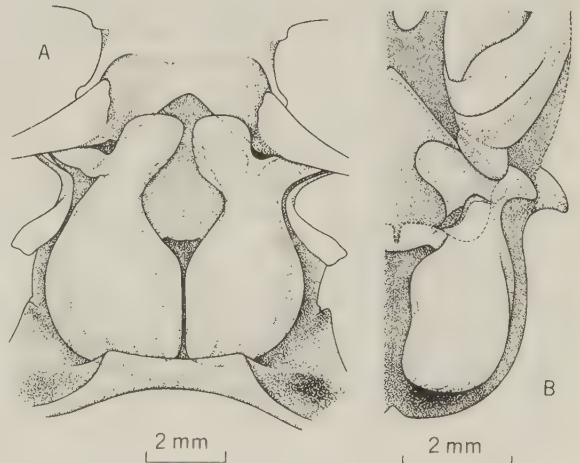


FIGURE 6.—*Trachypenaeus fuscina* sp. n. A. Seminal receptacles (dorsal view), ♀ 39.5 mm carapace length, off Salina Cruz, Oaxaca, México. B. Left seminal receptacle (ventral view), ♀ 31 mm carapace length, mouth of Río Suchiate, Chiapas, México.

Seminal receptacles derived from paired, deep invaginations at anterolateral extremities of sternite XIV. In impregnated females, seminal receptacles enclosing main component of spermatophores, consisting of thin walled sac containing subspherical masses of spermatozoa.

COLOR

Juveniles recently caught in inshore water of Oaxaca, México, light buff with brownish red suffusion; transverse, dark reddish brown bands on posterior part of abdominal somites; bands convex anteriorly, with widest portion on mid-dorsal line, extending ventrally and forming patch on posterior half of pleuron. Preserved adults (fresh ones not observed by me) with distinct dark abdominal bands, similar to those in juveniles.

SIZE

Largest specimen examined, ♀ 40.5 mm carapace length, about 150 mm total length, from off La Tapada, Chiapas, México, depth 7-9 m. Males smaller than females, largest observed ♂ allotype, 26 mm carapace length, 108 mm total length.

DISTRIBUTION

T. fuscina has been found in the Golfo de Tehuantepec, along the coasts of Oaxaca and Chiapas, México, and in the Golfo de Guayaquil, as far south as Tumbes, Perú. Although this species and *T. faoea* are sympatric in the Golfo de Guayaquil, apparently *T. fuscina* ranges farther north, since *T. faoea* has not been found along the southwestern coast of México.

REMARKS

T. fuscina is very closely related to *T. faoea*, but differs from it in several aspects—mainly in features of the telson (Figure 3A). In *T.*

fuscina the posteriormost pair of spines is longer, and fixed instead of movable, this being the only member of the genus *Trachypenaues* with immovable spines on the telson; the anterior three pairs of telsonic spines are stronger than in *T. faoea*, particularly those spines at the lateral base of the posteriormost pair; also the median sulcus is well marked as far as the base of the terminal portion of the telson. In *T. fuscina* the platelike base of the anterior part of sternite XIV in females (Figure 5A) is naked, and its lateral margins curve strongly posteromesially, giving rise to a deep emargination at the junction of the plate with the posterior part of the thelycum (sternite XIV). In males, the median plate of sternite XIII narrows anteriorly, usually tapering to a point (Figure 4F).

It should be pointed out that during copulation in this species, as well as in *T. faoea*, the male transfers to each seminal receptacle a very thin sac containing the spermatozoa, which are grouped into subspherical masses. These masses are not individually transmitted to the seminal receptacles as believed by Burkenroad (1934b), a phenomenon considered by him to be typical of the genus *Trachypenaues*.

ETYMOLOGY

Fuscina, L., = three-pronged fork—referring to the trifid appearance of terminal portion of telson.

Trachypenaues faoea LOESCH AND AVILA

FIGURES 3B, 4G, 5B

"CEBRA," "TIGRE," "INDIO," "CARABALI"

Trachypeneus faoe Lindner, 1957 [part], *nomen nudum*: 34, 35, 42, 43, 48, 49, 60, 61, 133, 134.

—U.S. Fish and Wildlife Service, 1962: 2, 6.—Crocker, 1967 [part]: 8, 19, 30, 39, 47, 57.

Trachypeneus faoea Loesch and Avila, 1964: 4-8, 16, 21, 24-28, fig. 8b, 13b.—Avila and Loesch, 1965: 3, 5, 6, 9, 10, 16, 19, 20, 23, 24, fig. 4b.

Trachypenaues face.—Food and Agriculture Organization of the United Nations, 1965: 10.

MATERIAL

Neotype.—♀, USNM 135398, Playas, Ecuador, January 7, 1964, Ortiz, 28 mm carapace length, 110 mm total length, ratio length of spine/width of terminal portion of telson = 0.25.

Panamá. 7 ♂ 21 ♀, USNM, Playa Bella Vista, March 20, 1955, M. D. Burkenroad. 3 ♀, USNM, Ensenada de Garachiné, Bahía San Miguel, 16 m, April 18, 1967, *Shimada* Sta. 14. 3 ♀, USNM, 4 km W of Punta Garachiné, 24 m, April 18, 1967, *Shimada* Sta. 78. Colombia. 7 ♀, USNM, Tortugas Grounds, S of Buenaventura, 9 m, September 19, 1969, L. W. Knapp, *Cacique* Sta. LK69-24. Ecuador. 22 ♀, USNM, Playas, January 7, 1964, Ortiz. 1 ♂ 4 ♀, USNM, Boca de Tenguel, April 14, 1966, Ansaldo.

TYPE MATERIAL

Loesch and Avila (1964) did not cite any material in particular; however, as the title of their work indicates, the specimens studied and illustrated in their keys were from Ecuador. Because of the close similarities between *T. faoea* and *T. fuscina* it seems mandatory that a neotype of *T. faoea* be selected, so that no confusion will arise as to the identity of the species of Loesch and Avila. Therefore, a neotype is here designated from a lot of females of *Trachypenaeus faoea* collected in Playas, Ecuador, which was identified and sent to me by the authors of the species. Inasmuch as the combination of specific morphological characters of this species have not been indicated previously, they are presented here.

"*Trachypeneus faoe*" first appeared in the literature in the report of a survey of the shrimp fisheries of Central and South America by Lindner (1957). This author grouped it with *T. byrdi* as one of the "larger zebra shrimp," noting the "blue-black" stripes on the abdomen and citing various other common names. Lindner, obviously, did not intend to describe the species, and did not include any specific character. He reported "*T. faoe*" in the shrimp fisheries of the American Pacific, from México to Perú, including El Salvador, Costa Rica, Panamá, Colombia, and Ecuador. Later (U. S. Fish and Wildlife

Service, 1962) the species was cited as one of "medium and small" shrimp found in the catches of Guatemala.

The original description of the species appeared in the keys to the commercial penaeid shrimps of Ecuador by Loesch and Avila (1964), and was mistakenly ascribed to Burkenroad. As stated by Avila and Loesch (1965), Burkenroad had planned to describe the species, but had not done so prior to the publication of their keys, nor has his description appeared since. *T. faoea* was distinguished from other penaeids found in the Ecuadorian catches on the basis of four morphological characteristics and color pattern. The following characters were cited: "No teeth on ventral portion of rostrum. . . . No well-developed dorsolateral sulcus on the posterior part of the last abdominal segment. . . . Backward-pointing dorsal spine on last abdominal segment only. . . . Telson not armed with lateral armature." The first two characters are shared by many species treated in their keys, and are actually supraspecific; the third character is common to all but one (*T. byrdi* Burkenroad) of the American Pacific *Trachypenaeus*, and the fourth is inaccurate: *T. faoea* does possess small lateral spines on the telson.

The color of the species was described as follows: "Tail section light anteriorly and dark posteriorly on each abdominal segment. . . . No distinctive patterns on second and third abdominal segments, each segment similarly colored with wide dark band covering $\frac{3}{4}$ the width of posterior part of each abdominal segment. More than $\frac{1}{2}$ of color of tail is dark (dark brown)." An entire animal and a separate abdomen were figured. In both illustrations the telson appears unarmed. The figure of the entire animal is accompanied by the rostral tooth formula, $\frac{6-7}{0}$. Of the characters cited, color is the only one that appears to be typical of the species, and it can only be applied to identification of fresh or recently preserved animals. It is unfortunate that circumstances prevented Loesch and Avila, who dealt with large numbers of specimens, from publishing a detailed description of *T. faoea*.

T. faoea is very closely related to *T. fuscina*, and except for the following, the above descrip-

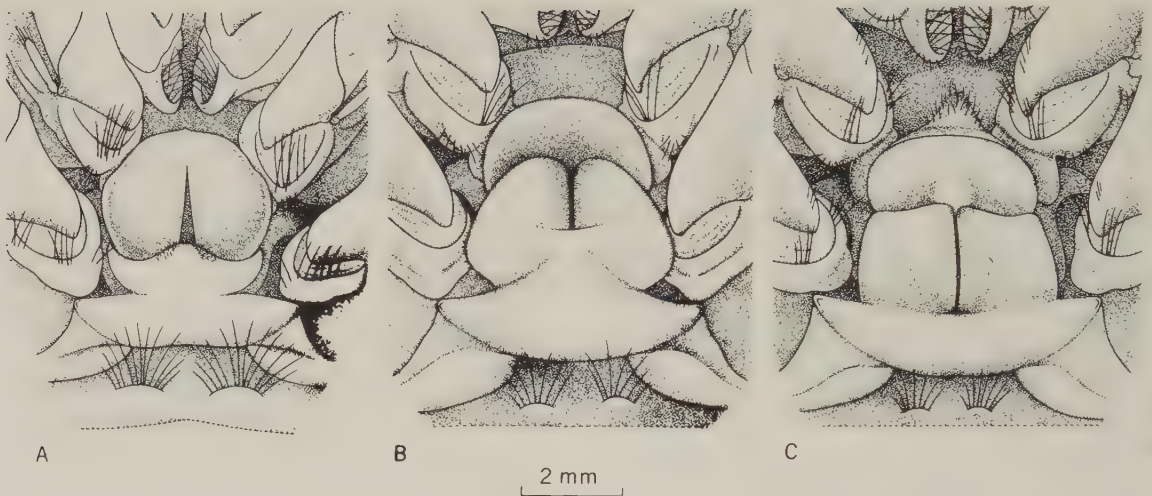


FIGURE 7.—Thelyca. A. *Trachypenaeus brevisuturæ* Burkenroad, ♀ 21.5 mm carapace length, off Zacapulco, Chiapas, México. B. *Trachypenaeus byrdi* Burkenroad, ♀ 27 mm carapace length, Golfo de Panamá, Panamá (syntype). C. *Trachypenaeus similis pacificus* Burkenroad, ♀ 25 mm carapace length, Archipiélago de las Perlas, Golfo de Panamá, Panamá (syntype).

tion of *T. fuscina* also applies to *T. faoea*: the armature of the telson, the shape and pubescence of the platelike base of the anterior part of sternite XIV in females, the marginal contour of the median plate of sternite XIII in males, and perhaps color.

In *T. faoea* the posteriormost of the four pairs of spines on the telson (Figure 3B) are movable instead of fixed, and shorter than those of *T. fuscina*, the ratio length of spine/width of terminal portion = 0.40 ($N\ 47: 0.75-0.20$); the other three pairs of movable spines, including those located at the lateral base of the posteriormost spines, are minute, actually microscopic. In *T. faoea* the median sulcus of the telson is deep anteriorly but hardly perceptible or indistinct posteriorly.

In males of *T. faoea* the median plate at the posterior margin of sternite XIII (Figure 4G) usually varies from subtrapezoidal (widest anteriorly) to suborbicular. In females, the structure of the thelycum (Figure 5B) is similar to that of *T. fuscina*; however, lateral margins of the platelike base of the anterior part of sternite XIV are almost straight, not or barely curving posteromesially, forming about 90° angles with

projections of the posterior part; furthermore, the lateral areas of that platelike portion are studded with rather densely set setae, the latter extending onto basal portions of the flaps.

Avila and Loesch (1965) described the color of recently preserved juvenile specimens of *T. faoea* as dark blue or purple with light uropods. They noticed that specimens preserved for 2 days in 5% Formalin exhibit a clear, horse-shoe shaped band on the first three abdominal somites, which is open anteriorly. These observations were based on material from Ecuador and differ from those made by me on *T. fuscina*. Recorded notes on color are so limited that conclusions in regard to differences in this character between the two species must await further observations throughout their ranges.

DISTRIBUTION

Although this species has been reported from as far north as the coasts of Guatemala, El Salvador and Costa Rica, the collections available to me are limited to the area from Panamá to Ecuador. Examination of additional samples of

"cebra," or "tigre" shrimp from Central and South America will be necessary to establish the actual distribution of *T. faoea* as well as that of *T. fuscina*, both known by the same common names.

With the discovery of an additional species of *Trachypenaeus* in the American Pacific, it seems appropriate to present a key to the five members of the genus occurring in the region, together with the range of each.

KEY TO THE AMERICAN PACIFIC SPECIES OF *Trachypenaeus*

1. Carapace with longitudinal suture short, not extending to hepatic spine. Third maxilliped lacking spine on basis; first pereopod bearing spine on ischium. Thelycum with median plate armed with small anteromedian spine (clearly distinct in juvenile, barely perceptible in adult); anterior part of sternite XIV shorter than posterior part, and not produced as paired flaps (Figure 7A). Petasma with dorsal spinelike projection near apex of horn *T. brevisuturae* Burkenroad
(From off Punta Arenas, Golfo de California, to El Salvador).

Carapace with longitudinal suture long, extending posteriorly beyond hepatic spine. Third maxilliped bearing spine on basis; first pereopod lacking spine on ischium. Thelycum with median plate lacking anteromedian spine; anterior part of sternite XIV longer than posterior part, and produced as paired flaps. Petasma without projection near apex of horn 2

2. Spine present on posterior end of middorsal carina of last two, three, or four abdominal somites. Telson unarmed. Thelycum with slit between flaps never reaching posterior part of sternite XIV, and with median plate short, not reaching gonopores (Figure 7B) *T. byrdi* Burkenroad
(From Guatemala to Golfo de Guayaquil)

Spine present only on posterior end of middorsal carina of last abdominal somite. Telson armed with lateral spines. Thelycum with slit between flaps reaching posterior part of sternite XIV, if not, with median plate long, reaching, or almost reaching, gonopores . . . 3

3. Rostral teeth 7 to 10, usually 8 or more. Telson with proximal triangular patch of long setae on each side of median sulcus. Thelycum with median plate not excavated, short, and protruding ventrally on midportion. Anterior part of sternite XIV lacking platelike base and bearing subrectangular flaps, extending only to posterior part of median plate (Figure 7C) *T. similis pacificus* Burkenroad
(From Bahía Concepción, Golfo de California, to Tumbes, Perú)

Rostral teeth 6 or 7. Telson lacking proximal, triangular patches of long setae. Thelycum with median plate strongly excavated. Anterior part of sternite XIV with platelike base, and bearing paired, much elongate, tonguelike flaps, extending well beyond midlength of median plate 4

4. Telson with posteriormost pair of lateral spines fixed. Thelycum with platelike base of anterior part of sternite XIV lacking setae on each side, its lateral margins strongly curving posteromesially, forming deep emargination with posterolateral projections (Figure 5A) *T. fuscina* sp. n.
(Golfo de Tehuantepec and Golfo de Guayaquil)

Telson with posteriormost pair of lateral spines movable. Thelycum with platelike base of anterior part of sternite XIV setose, its lateral margins almost straight, forming about 90° angles with posterolateral projections (Figure 5B) *T. faoea* Loesch and Avila
(From Guatemala to Golfo de Guayaquil)

ACKNOWLEDGMENTS

In addition to the collections in the United States National Museum (USNM), Smithsonian Institution, material was obtained from the Instituto de Biología, Universidad Nacional Autónoma de México (IBUNM), through Alejandro Villalobos F. and Jorge A. Cabrera, and the Instituto Nacional de Investigaciones Biológico-Pesqueras, Secretaría de Industria y Comercio de México (INIBP), through Amin Zarur and Héctor Romero. I wish to thank the above mentioned persons for their assistance and cooperation. The two collections from México have been divided, one part deposited in the corresponding Mexican institution and the other at the USNM. I am indebted to Quinto Avila and Harold Loesch for critical material from Ecuador, and to Leslie W. Knapp for making available to me specimens from Colombia. Horton H. Hobbs, Jr., Fenner A. Chace, Jr., and Bruce B. Collette offered critical comments that considerably improved the manuscript. The illustrations are by the artist María M. Diéguez, National Marine Fisheries Service Systematics Laboratory.

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ARTIFICIAL RIPENING OF MAATJES-CURED HERRING WITH THE AID OF PROTEOLYTIC ENZYME PREPARATIONS

T. M. RITSKES¹

ABSTRACT

For manufacturing maatjes-cured herring, the herring caught in the North Sea or Irish Sea is suitable only during months when the proteolytic activity of the appendices pyloricae is sufficiently high. For instance, only North Sea herring caught from May to July has sufficient proteolytic activity for the manufacture of a well-ripened product. For herring caught in other areas, this period may differ considerably. The present work shows that it is possible to use herring caught in other seasons if protease preparations are added to the fish together with salt. The herrings were examined organoleptically, whereas the brines were examined by chemical analysis and by chromatography on Sephadex G-25. No significant differences were found between naturally ripened herring and herring cured by the aid of enzyme preparations. The lipase content of the preparation should be low enough in order to avoid the formation of a fatty acid taste in the cured fish.

Maatjes-type cured herring, the fish product that has been made for centuries aboard Dutch fishing vessels, is usually made from North Sea herring (*Clupea harengus* L.), and in latter years from Irish Sea herring as well.

After being caught, the herring is gibbed and salted promptly. "Gibbing" means the removal of some of the intestines through an incision below the left gill. It is essential that, at this procedure, the appendices pyloricae are left in the fish; according to Luijpen (1959) this organ plays an important role in the formation of the characteristic organoleptic properties of the product. The particular taste and the soft consistency are due to the action of proteolytic enzymes from the appendices pyloricae on the fish flesh.

Since the content of proteolytic enzymes in the fish varies with the seasons, the fish is suitable for the manufacture of maatjes-cured herring only for a few months. Other variables like age and size of the fish or its source may also influence the protease content and in this way restrict the suitability of herring for the maatjes-curing process. For these reasons, there is a demand for a manufacturing method

which is less dependent upon the protease activity of the appendices pyloricae.

The aim of our investigation was, therefore, to find the conditions for obtaining an acceptable maatjes-cured herring by the addition of protease preparations. In this way a product with the desired organoleptic properties might be manufactured from herring which have relatively inactive appendices pyloricae and which consequently cannot ripen in a natural way.

MATERIALS AND METHODS

Artificially ripened herring was made by adding protease preparations and salt to gutted fresh or frozen herring. The enzyme preparations were mixed with the salt before salting the herring in the usual way, viz. mixing the herring with a certain quantity of dry salt. Shortly after the addition of salt, a brine is formed which covers the fish entirely. After storage from 7 to 31 days the fish was examined organoleptically and the brines chemically. In most cases the enzyme-treated herring was compared with naturally ripened herring and with eviscerated ("gutted") herring to which no enzyme preparations were added.

The purpose of the chemical analyses was to gather information about possible differences

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between the natural and the artificial ripening process. These analyses included: assay of total soluble nitrogen by the Kjeldahl method, protein determination by the biuret method, and determination of amino nitrogen (as a rough measure for the amino acid content). Assuming that the soluble matter is evenly distributed between the fish and the brine formed, most of the analyses were carried out in the latter.

Some of our experiments are described below. Other experiments gave similar results and are omitted here for ease of survey.

NATURAL RIPENING PROCESS

Frozen maatjes herring that had been caught in the North Sea at the end of May were used. The protein content was 16.4% and the fat content 13.8%. Herring in this stage is normally used for the preparation of maatjes-cured herring.

The protein content was determined by the Kjeldahl method; for the fat determination, the Bligh and Dyer (1959) method was used in a modification according to Ederzeel and Ritskes (1966).

Part of the herring was gibbed and part was gutted. One part of salt was added to 20 parts of herring (light salting). The fish was kept at 3° C for 1 week.

In order to remove proteins which are assumed to be of less importance in the study of the ripening process, the brines were heated for about 15 min at 80° C and then filtered warm over a fluted filter paper. All analyses were carried out with these clarified brines. In this experiment, these analyses included a Kjeldahl nitrogen determination, a measurement of the biuret value according to Strickland, Freeman, and Gurule

(1961) and a determination of the amino nitrogen content according to the method of the Dutch Food Law (1963). This latter method is based upon two alkalimetric titrations to different end points. The salt content in the brines was determined by the Volhard method.

In order to elucidate the protein breakdown process during ripening, gel chromatography of the brine was applied; 0.8 ml of a clarified brine was chromatographed on a Sephadex G-25 column² (length 44 cm, diameter 1.44 cm; fraction volume 3.4 ml, elution rate 19.8 ml/h), distilled water being used as an eluant. In each fraction the biuret value and the absorbance at 280 nm (nanometers or millimicrons) were measured. The results of the organoleptic evaluations and the chemical analyses are shown in Table 1. The results of gel chromatography are plotted in Figures 1 and 2. As could be expected, the values found in the brine of the gibbed herring were considerably higher than in that of the gutted herring.

TESTING OF TWO ENZYME PREPARATIONS

Frozen spawning herring, caught at the end of August in the North Sea were used. The protein content was 17.6% and fat content 18.2%.

Two enzyme preparations, Pr 8 and TG 21/63, were tested. Their proteolytic activities were determined according to Anson (1938), with denatured hemoglobin as a substrate. Lipolytic activity was determined on olive oil according to Marchis-Mouren, Sarda, and Desnuelle

² Reference to trade names in this publication does not imply endorsement of commercial products by the National Marine Fisheries Service.

TABLE 1.—Results of organoleptic evaluation of naturally ripened maatjes-cured herring and of analyses of brine in which herring ripened.

Variation	Organoleptic evaluation of the herring		Analyses of the brine ¹		
	Flavor	Texture	Biuret value (Eso × 1000 × dilution factor)	Total N (mg N/ml)	Amino N (% w/v)
Gutted	Nontypical	Moderately firm	900	4.4	0.133
Gibbed	Good maatjes-flavor	Optimal (soft)	1720	6.9	0.255

¹ Salt content of brine was 10.5 ± 0.5% w/v.

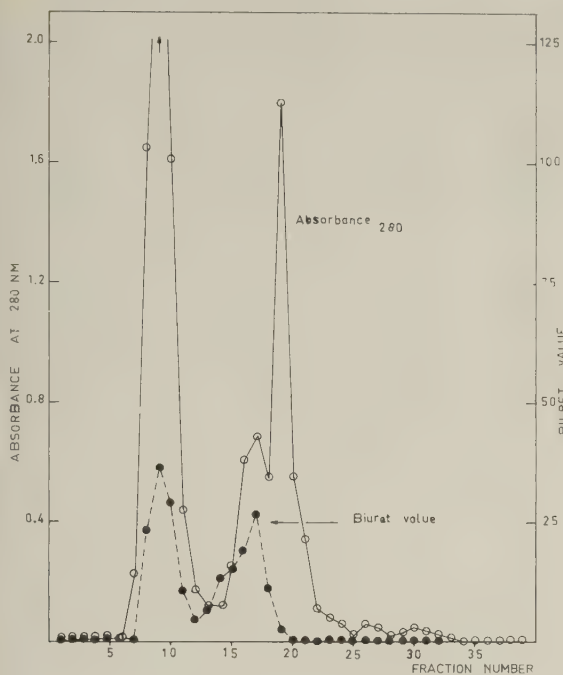


FIGURE 1.—Chromatogram of the clarified brine of gutted maatjes herring on Sephadex when herring were ripened naturally.

(1959). Pr 8 had a proteolytic activity of 5.3×10^{-4} U/mg and a lipolytic activity of 0.04 Desnuelle units/mg; for TG 21/63, these values were 9.1×10^{-4} and 18.7, respectively. These preparations, like all others used in this study, were supplied by N. V. Organon, Oss, the Netherlands. Details concerning these preparations are summarized in Table 2.

The proteolytic activity of the preparations was found by measuring the caseinolytic activity at pH 7.5 and 35° C (Ruyssen, 1969). The NF-pancreatin reference standard was used (see National Formulary XIII, 1970, p. 514). The potency of the protease preparations is expressed in terms of the minimum activity required by NF XIII. The lipase activity was measured by potentiometric titration of fatty acids hydrolyzed in an olive oil emulsion at pH 8.0 (Marchis-Mouren, Sarda, and Desnuelle, 1959). The International F.I.P. standard for pancreas lipase was used as a standard (Ruyssen, 1969). One

unit forms 1 μ mol fatty acid per minute under the conditions of the assay.

In order to eliminate the effect of the intestinal enzymes, the fish was gutted before the enzyme preparations were added.

One part of salt was added to seven parts of herring (w/w). The fish was kept at 15° C for 3 weeks. Analyses were taken after 1, 2, and 3 weeks; organoleptic evaluation took place after 2 weeks.

Four variations were tested: (a) gutted; (b) gibbed; (c) gutted and 2.0 g of Pr 8 per kg herring added; (d) gutted and 1.2 g of TG 21/63 per kg herring added.

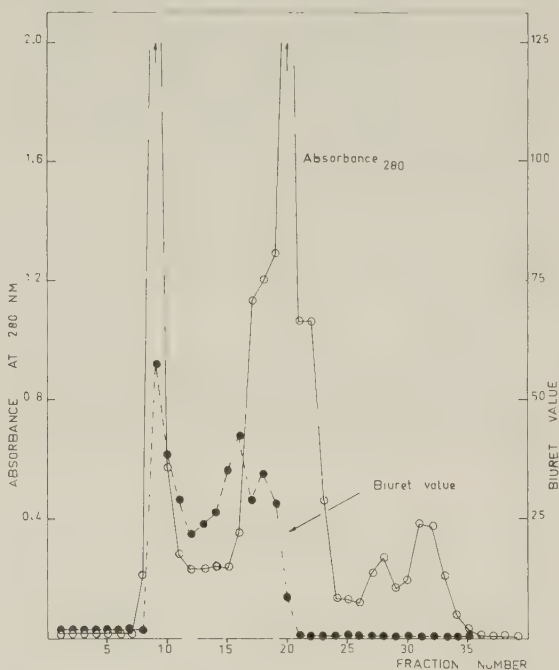


FIGURE 2.—Chromatogram of the clarified brine of gibbed maatjes herring on Sephadex when herring were ripened naturally.

TABLE 2.—Enzyme preparations used in the experiments.

Code No.	Origin and description
TG 21/63	Porcine pancreas powder
Pr 8	Porcine pancreas protease mixture with low lipase activity
Pr 34	Pancreas protease from sheep
Pr 35	Beef pancreas protease mixture
Pr 11/66	Same quality as Pr 8
W	Beef pancreas protease concentrate
CH 32/67 A	Same quality as Pr 8

TABLE 3.—Results of organoleptic evaluations and chemical analyses from experiment using two enzyme preparations.

Variation	Organoleptic evaluation		Analyses of the brine ¹					
			Biuret value			Total nitrogen		
	Flavor	Texture	After 1 week	2 weeks	3 weeks	After 1 week	2 weeks	3 weeks
Gutted	Salty; not ripened	firm	856	1,105	1,305	5.1	6.0	6.0
Gibbed	Not fully ripened	firm	--	1,320	--	--	8.1	--
Gutted + Pr 8	Ripened flavor	soft	2,620	3,670	4,320	10.0	11.8	13.4
Gutted + TG 21/63	Ripened flavor but with an unpleasant fatty acid flavor	soft	2,740	3,950	4,680	10.4	13.1	15.0

¹ The salt content in the brine was $20 \pm 2\%$ w/v.

The brine was analyzed as described earlier, but with the omission of the amino-N determination.

The results of the organoleptic evaluations and the chemical analyses are shown in Table 3. They show that, by the addition of enzyme preparations to gutted herring, it is possible to obtain a product with "maatjes-cured" organoleptic properties. A high lipolytic activity, however, seems to be undesirable.

Both the biuret value and the total nitrogen content in the brine increase during the ripening period. There seems to be a relation between the values found and the degree of ripening.

ARTIFICIAL RIPENING OF FRESH SPENT HERRING WITH ENZYME PREPARATIONS LOW IN LIPASE ACTIVITY

After the herring has spawned, the uptake of food stops and the fat content decreases gradually to relatively low values, e.g., 5 to 8%. Since the proteolytic activity of the appendices ptyroicae is low in this period, the fish as such is unsuitable for the manufacture of maatjes-cured herring.

Fresh, spent herring, caught in February in the Irish Sea were used. The protein content was 17.1% and the fat content 5.2%.

Activity of the enzyme preparations tested is shown in Table 4.

In view of the results obtained in the experiment on spawning herring, the proteolytic activity in the artificial maatjes-cured herring was reduced in this experiment.

Six variations were tested: (a) gutted; (b)

gibbed; (c, d, e, f) gutted and added respectively: 1.0 g Pr 34, 1.0 g Pr 35, 1.0 g Pr 11/66 and 55 mg W per kg of fish. The fish was kept at 3° C for 3 weeks. One part of salt was added to 10 parts of herring.

The brine was analyzed as described earlier. In analyses of the herring, 30 g of ground herring fillets were homogenized with 100 ml of water in an Ultra-Turrax mixer. The mixture was heated to 80° C and, after cooling to room temperature, filtered through fluted filter paper. In the extract thus obtained, the same analyses as earlier described for the brine were carried out. Gel chromatography was performed as described earlier.

The results of the organoleptic evaluations and the chemical analyses are shown in Table 5.

The brines of the variations "gutted" and "gutted + Pr 35" were chromatographed. The results are plotted in Figures 3 and 4.

With the preparations Pr 35 and Pr 11/66, an acceptable product was obtained. W gave rise to a good texture but developed less flavor. Pr 34 caused some off-flavor, probably because of the comparatively high lipolytic activity. It seems to us that a lipolytic activity lower than 1 U/mg is desirable for a preparation which has a protease activity equivalent to the minimum activity required by NF XIII.

TABLE 4.—Proteolytic and lipase activities of enzyme preparations.

Enzyme preparation	Proteolytic activity according to NF XIII	Lipase activity: Desnuelle U/mg
Pr 34	1.6 × NF	1.8
Pr 35	1.0 × NF	0.2
Pr 11/66	3.0 × NF	0.2
W	20 × NF	0.6

TABLE 5.—Results of organoleptic evaluations and chemical analyses from experiment with the artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

Variation	Organoleptic evaluation		Chemical analyses					
	Flavor	Texture	In the brines ¹			In the extracts		
			Biuret value	Total N	Amino N	Biuret value	Total N	Amino N
Gutted	Nontypical	Moderately firm	1,345	5.6	0.138	192	0.55	0.017
Gibbed	Nontypical	Moderately firm	1,665	7.8	0.178	---	---	---
Gutted + Pr 34	Fair, but with some off-flavor	Soft	2,690	8.8	0.255	---	---	---
Gutted + Pr 35	Fair	Too soft	2,900	9.1	0.252	---	---	---
Gutted + Pr 11/66	Fair	Soft	2,355	7.3	0.210	487	1.29	0.036
Gutted + W	Nontypical	Soft	2,460	8.2	0.224	---	---	---

¹ The salt content in the brines was 12 ± 1% w/v.

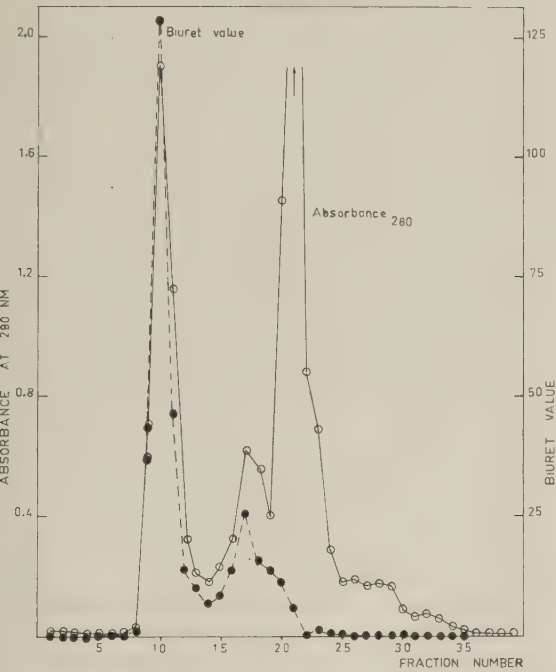


FIGURE 3.—Chromatogram of the clarified brine of gutted herring, without the addition of enzymes on Sephadex G-25, in experiment on artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

There exists a relation between the texture of the herring and the biuret value found in the brine. Between texture and total amount of nitrogen in the brine no apparent relation was shown. The ratio total N to amino N is about the same in the brine and in the herring extract, indicating that analysis of the brine give information about the ripening process in the herring.

The chromatograms show that, in the brine of the ripened herring, the amount of biuret-positive material with larger retention times has increased. This indicates that the amount of small protein fragments increased. The chromatographic pattern of the brine of an artificially ripened herring (Figure 4) does

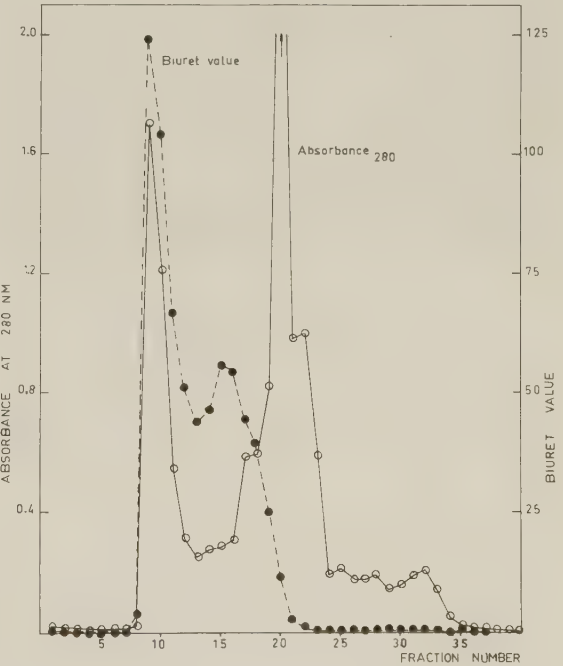


FIGURE 4.—Chromatogram of the clarified brine of gutted herring, with the addition of the enzyme Pr 35, on Sephadex G-25, in experiment on artificial ripening of fresh spent herring with enzyme preparations low in lipase activity.

not differ essentially from that of the gibbed herring brine (Figure 2). With the methods used, no obvious difference was found between the natural and the artificial ripening process.

THE ARTIFICIAL RIPENING OF LEAN HERRING AND ITS RELATION TO CHANGES IN THE RESULTS OF BRINE ANALYSIS

Fresh herring caught in the North Sea at the beginning of April were used. The protein content was 16.7%; fat content, 10.6%.

Two variations were studied: (a) gutted; (b) gutted and with 1.0 g of Pr 35 per kg of herring added. One part of salt was added to 10 parts of herring. The fish was kept at 3° C. Samples for analysis were taken after 11, 17, 24, and 31 days. Organoleptic evaluation was carried out after 24 days.

The brine was analyzed as described earlier.

After 24 days at 3° C, organoleptic evaluation showed: variation (b) had a soft texture and a well-ripened flavor, whereas variation (a) was still firm and had less flavor.

Results of chemical analysis of the brine are plotted in Figure 5 and summarized in Table 6.

The salt content in the brines was $16 \pm 2\%$ w/v.

During the ripening process there is a steady increase in biuret value, total N, and amino N in the brine. This finding demonstrates again the relation between these values and the degree of ripening.

THE ARTIFICIAL RIPENING OF FRESH LEAN HERRING WITH DIFFERENT AMOUNTS OF A PROTEASE PREPARATION

Fresh spent herring caught near the Hebrides in August was used. This herring contained 17.4% of protein and 8.2% of fat.

In this experiment an enzyme preparation CH 32/67 A was tested, with a proteolytic activity of 1.93 times NF XIII units and a lipolytic activity of 0.3 Desnuelle units per mg.

One part of salt was added to 10 parts of fish. Six variations were tested: (a) gutted; (b) gibbed; (c, d, e, f) gutted and 0.5, 1.0, 2.0, and 5.0 g of CH 32/67 A added, respectively. The fish was kept at 3° C for a month, then evaluated and analyzed.

The brine was analyzed as discussed earlier. Results are shown in Table 7.

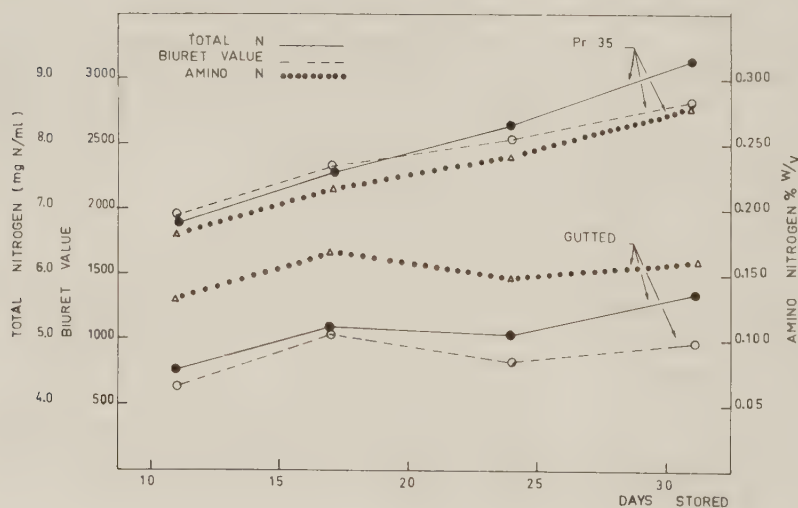


FIGURE 5.—Results of analyses of clarified brines of gutted herring with and without addition of Pr 35, in experiment on the artificial ripening of lean herring and its relation to changes in the result of brine analysis.

TABLE 6.—Chemical analyses of the brines¹ in experiment on artificial ripening of lean herring.

Brines	Gutted	Gutted + Pr 35
Biuret value:		
after 11 days	625	1,915
after 17 days	1,010	2,320
after 24 days	840	2,520
after 31 days	960	2,820
Total N:		
after 11 days	4.5	6.8
after 17 days	5.2	7.6
after 24 days	5.1	8.3
after 31 days	5.7	9.3
Amino N:		
after 11 days	0.131	0.183
after 17 days	0.165	0.215
after 24 days	0.146	0.238
after 31 days	0.160	0.282

¹ The salt content in the brines was $16 \pm 2\%$ w/v.

An addition of 0.5 g of CH 32/67 A per kg of herring is too low to obtain a well-ripened product under these conditions. An addition of 1.0 g per kg, however, seems somewhat too high. A suitable dose is probably the amount of protease that corresponds with a proteolytic activity of 2000 mg-eq NF XIII powder per kg of herring.

In the brines (b) and (d) the same amino-N contents were found, but the biuret value in (d) was considerably higher than in (b). This suggests that the action of the enzyme preparation is particularly the breakdown of muscle protein to larger fragments, whereas the endogenous enzymes show more peptidase activity. This is in accordance with the observation that an overdose of the enzyme preparation results in a very soft rather than in a strong-tasting herring.

SUMMARY

The addition of a certain quantity of a protease preparation to herring which is unsuitable for maatjes curing has a favorable effect on both flavor and texture of the herring.

An addition of 2000 mg-eq NF XIII powder per kg of herring is proposed. Doubling or halving this amount had a pronounced effect on the organoleptic properties of the cured herring. The lipase activity should be low.

In the brines the biuret value, the total N content and the amount of amino N increase gradually. There appears to be a distinct relation between the biuret value in the clarified brine and the consistency of the herring; the same is true for the amino N content in the brine and the flavor of the herring.

The ratio biuret value: amino N content found in the brine was higher for artificially ripened herring than for the naturally ripened product. This finding indicates that the enzyme preparations are poorer in peptidase activity than the appendices pyloricae from the herring.

The protein breakdown in the artificially ripened herring, however, does not seem to be essentially different from that in the naturally ripened herring. The patterns obtained by chromatography of the brines over Sephadex G-25 did not show any essential difference.

ACKNOWLEDGMENTS

The author wishes to thank A. Ruiter and L. G. Heeringa for their valuable assistance in writing the manuscript.

TABLE 7.—Results of organoleptic evaluations and chemical analyses from experiment on artificial ripening of fresh lean herring with different amounts of a protease preparation.

Variation	Organoleptic evaluation		Chemical analyses of brines ¹		
	Flavor	Texture	Biuret value	Total N	Amino N
Gutted	Salty; no ripened flavor	Firm	1,300	5.4	0.195
Gibbed	Nontypical	Moderately firm	1,640	9.0	0.328
Gutted + 0.05% CH 32/67 A	Fair	Moderately soft	2,320	8.0	0.274
Gutted + 0.10% "	Fair	Optimal (soft)	2,875	9.2	0.334
Gutted + 0.20% "	Strongly ripened	Too soft	3,210	10.7	0.398
Gutted + 0.50% "	Not tasted	Too soft	3,650	11.9	0.465

¹ The salt content in the brines was $12 \pm 1\%$ w/v.

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LABORATORY STUDIES OF PREDATION BY MARINE COPEPODS ON FISH LARVAE

KURT LILLELUND^{1, 2} AND REUBEN LASKER³

ABSTRACT

A variety of marine copepods have been shown to fatally injure or capture and ingest young anchovy larvae in the laboratory. *Labidocera jollae*, *L. trispinosa*, and *Pontellopsis occidentalis* (family Pontellidae), species common to surface waters of the California Current, are effective predators of larval fish. The copepods can be attracted by the vibrations of the larval tail beat and react by biting or capturing the fish larvae. Cruising speeds for these copepods varies from 1.5 to 4 body lengths per second which, coupled with continuous swimming behavior, results in extensive searching by the copepod for prey. In laboratory experiments, when the ratio of larval fish prey to *L. jollae* female individuals was low (<10:1 in 3500 ml), all of the larvae were killed in 24 hr where "killing" refers to both capture-ingestion and biting resulting in a fatality. If the ratio was higher, killing increased but rarely reached 100% mortality of the larvae. *L. trispinosa* males and females never killed all the larvae offered to them in 24 hr in 3500 ml although more larvae were killed as the number offered was increased. Increased swimming and escape ability developed as anchovy larvae became older and were not caught or bitten as effectively as younger ones by copepods. However, killing of larvae by *P. occidentalis* was unaffected by the age of the larvae up to 3.5 days old.

When *Artemia salina* nauplii were substituted for larval fish as prey for *L. trispinosa*, the amount of grazing was proportional to the ratio of nauplii to copepods. If the number of *Artemia* nauplii was less than 11-14/liter per copepod in 3500 ml all the nauplii were killed in 24 hr. When the density of nauplii was increased, more were killed but never all of them. In experiments where nauplii and yolk-sac larvae were offered together as prey the mortality of the larvae due to predation declined in proportion to the increase in the number of nauplii provided.

Caloric requirements were calculated from oxygen consumption measurements and showed that only 1 to 4 anchovy larvae are required per day per copepod to satisfy the metabolic needs of *Labidocera*, depending on the species and the sex. This number is far less than can be killed or captured if the density of larvae is high enough.

In large (140 cm) vertical cylinders larvae and *L. trispinosa* were distributed within 25 cm of the surface in the dark. Data are presented which show that *L. trispinosa* and anchovy larvae also co-occur in the upper few meters under the surface of the sea. This is probably also true for *P. occidentalis* and *L. jollae*. No data are yet available on the relative density of predatory copepods and fish larvae where they co-occur or their possible predator-prey interactions in the sea.

Huge mortalities of larval fish are known to occur in the sea. From the time of Hjort (1914) these have been attributed mainly to the lack of the proper food when the larvae begin to feed (see review by Blaxter, 1969). Undoubtedly other biotic and abiotic factors are also involved

in larval fish mortality, but comparatively little work has been done to measure their effect. Among the possible causes of larval-fish mortality, predation by other zooplankters may be an important factor. Freshwater aquarists have known for some time that copepods must be eliminated from fish rearing tanks or high mortalities of young larvae or fry will occur (Davis, 1959). Lillelund (1967) reviewed the literature pertaining to predation by freshwater copepods on fish larvae and described the predatory behavior of cyclopoid copepods as he observed them

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² This research was supported in part by a Grant from the Deutsche Forschungsgemeinschaft.

³ National Marine Fisheries Service, Fishery-Oceanography Center, La Jolla, Calif. 92037.

in the laboratory. Zooplankters in general and copepods in particular have been seen to devour marine fish larvae. For example, in an early study on the rearing of marine fish, Garstang (1900) noted that the harpacticoid copepod *Idya furcata* (= *Tisbe furcata*) was a larval-fish predator. Subsequently Lebour (1925) from her examination of living marine plankton, concluded that a variety of zooplankters eat fish larvae and noted that "many jellyfishes and *Pleurobrachia* besides *Sagitta* and *Tomopteris* will readily eat small fishes." Lebour also illustrated the capture of an anglerfish larva (*Lophius piscatorius*) by the copepod *Anomalocera pattersoni* (family Pontellidae).

Despite this information on the predatory behavior of copepods, there is virtually no behavioral information available on the ability of marine copepods to capture and ingest or fatally injure fish larvae, although many marine copepods are known to be carnivorous (Gauld, 1966) and other incidental reports of copepods predatory on marine fish larvae have been made (Wickstead, 1965; Petipa, 1965). Furthermore, the possible importance of predation on fish larvae as it affects the determination of year class strength through larval fish mortality has been generally ignored, probably because of the lack of pertinent quantitative laboratory and field information.

In this study we present the results of experiments designed to measure quantitatively the ability of three pontellid marine copepods, *Labidocera trispinosa*, *L. jollae*, and *Pontellopsis occidentalis*, to capture or fatally injure larvae of the northern anchovy, *Engraulis mordax*, an important commercial fish of the California Current. The behavior of copepods and larvae which bears on the susceptibility of the latter to predation is also described in detail.

METHODS

Copepods and anchovy eggs were captured with a 0.5-m-mouth-diameter plankton net (0.333-mm mesh) towed at the surface in coastal water off San Diego, Calif., between March and August 1970. The copepods were separated

from other plankton with a large bore pipette on shipboard, diluted with surface water in liter jars, and kept at sea water temperature (about 15° C) in an insulated chest until returned to the laboratory.

All experiments were performed in 3500-ml beakers in the dark because *Labidocera* were phototactic and attracted to the light source and *Pontellopsis* was inhibited in its attacks on larvae in the light. In an earlier study on freshwater cyclopoid copepods, Lillelund (1967) used a constant level, continuous flow device which we used also for maintaining marine copepods in good condition in the laboratory (Figure 1). However, it was more convenient to do all predation experiments in static water over a 20- to 24-hr period, since the copepods we investigated swim continuously throughout the small volume (3500 ml), obviating the need for continuously circulated water. A constant temperature of 18° C was maintained in the beakers by placing them in a running seawater bath.

Mortality of the larvae was measured by taking the difference between the number of larvae at the beginning of the experiment and those remaining alive at the end. Some mortality not associated with predation always occurred, hence control vessels containing larvae alone were always provided and the results of experiments corrected for larvae dead of other causes. In all experiments, this natural mortality never exceeded 10%.

Anchovy eggs were sorted in the laboratory according to their stage of development and newly hatched larvae were used as prey in the predation experiments. When older larvae were required they were reared according to the method of Lasker et al. (1970), except that the rotifer *Brachionus plicatilis* was substituted for snail veligers as larval-fish food (Theilacker and McMaster, in press).

Oxygen consumption measurements were made by Warburg manometry. Usually 18 to 22 copepods were put into 3 ml of seawater in a Warburg flask and oxygen uptake monitored for 8 hr at 18° C. Dry weight of individual copepods was measured with an electrobalance to $\pm 2 \mu\text{g}$.

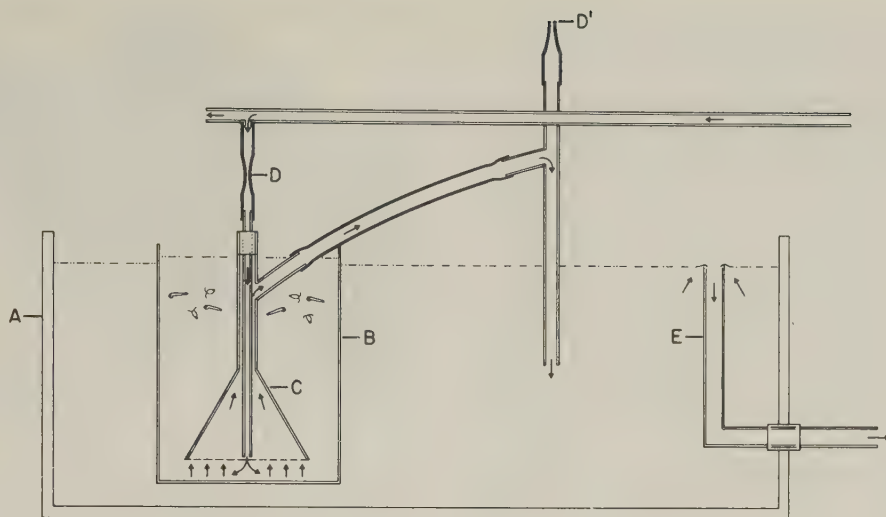


FIGURE 1.—Constant level device for maintaining copepods and fish larvae. The temperature of the 3500-ml beaker (B) was kept constant with running seawater in a water table (A) whose capacity was 610 liters. Plankton netting (0.333-mm mesh) was held over the mouth of the 500-ml funnel (C) with a section of bicycle tire tube. Rubber tubing showing constrictions as D and D' had screw-type clamps to regulate flow. E is the drain. Note that the level of seawater is higher in the beaker than in the bath. The drawing is not to scale.

MARINE COPEPODS CAPABLE OF KILLING ANCHOVY LARVAE

Before choosing *Labidocera* and *Pontellopsis* for experimental work we tested a number of copepod species for their ability to capture or fatally injure newly hatched yolk-sac larvae of the northern anchovy. For each test, five fish larvae were isolated in 200 ml of seawater in a Petri dish at room temperature (20° C) usually with two or three copepods of a particular species to be tested. Of the local copepod species observed, the following fatally injured anchovy larvae by biting them or captured and ingested them:

Acartia dana and *A. tonsa*
Euchirella rostrata and *E. sp.*
Labidocera jollae and *L. trispinosa*
Pleuromamma borealis
Pontellopsis occidentalis
Euchaeta acuta

Euaetideus acutus
Candacia bipinnata

Because the two species of *Labidocera* listed above are common to waters adjacent to San Diego and were readily available, most of our experimental work was done with them. When *Pontellopsis occidentalis* became abundant, we also collected some information about its predatory behavior relative to fish larvae.

Although capture and ingestion of fish larvae was commonplace under laboratory conditions, it is rare to find a copepod with a captured fish larva in Formalin-preserved plankton. This may be the result of the Formalin preservation common on shipboard which, we have observed, usually causes copepods to drop larvae.⁴

⁴ We have found that if a copepod has captured a larva it will retain the larva if both are transferred together to a slight melted depression in an ice cube with a pipette and preserved with a drop of 3% Formalin.

SWIMMING AND FEEDING BEHAVIOR OF *Labidocera*

REACTION TO THE LARVAL TAIL BEAT; BITING AND INGESTION OF LARVAE

We noted that individual *Labidocera* ignored motionless fish larvae or floating eggs. However, when a larva beat its tail in the close vicinity of a swimming labidoceran, the copepod swam immediately toward the beating tail and grasped the larva. The tail beat of the larva was often stimulated by the chance touch of a copepod's antenna. Figures 2a and 2b show a *L. jollae* female which caught a 3-day-old anchovy by the tail and partially ingested it. Figure 3 shows another larva caught behind the head

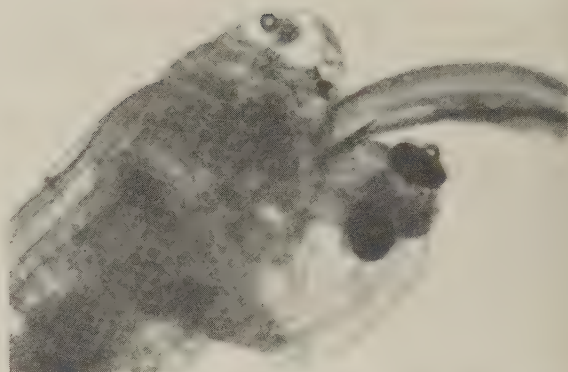


FIGURE 3.—The head of a *Labidocera jollae* female showing a newly captured 3-day-old anchovy larva caught behind the head.

by another female. Response to the larval tail beat is typical of all the copepods we have observed which attack fish larvae.

Often a copepod would capture, then drop a larva, inflicting a wound by biting the thin epithelium. The anchovy larval skin is only about 2 to 3 μ thick in the finfold and tail regions and appears to be easily injured. In every instance, a bite which damaged the larval skin resulted in the death of the larva. Therefore, in our experiments mortality due to a copepod was the result of either actual capture and ingestion of a larva or biting that resulted in damage fatal to the larva. Hence, the number of larvae reported as "killed" in an experiment is the sum of fatalities due to biting and the number of larvae actually ingested. For both sexes of each *Labidocera* species the time of ingestion of anchovy larvae varied between 6 and 25 min. In one instance an *L. trispinosa* male caught and completely consumed two larvae in 1 hr. If we increased the number of larvae to six or more in a 200-ml Petri dish containing two copepods, mortality through biting alone increased and the time during which a larva was held by a copepod varied from a few seconds to minutes. For example, an *L. jollae* female attacked six larvae in 50 min. The individual larvae were held only 10 to 60 sec and not ingested. All six larvae died subsequent to the attack.

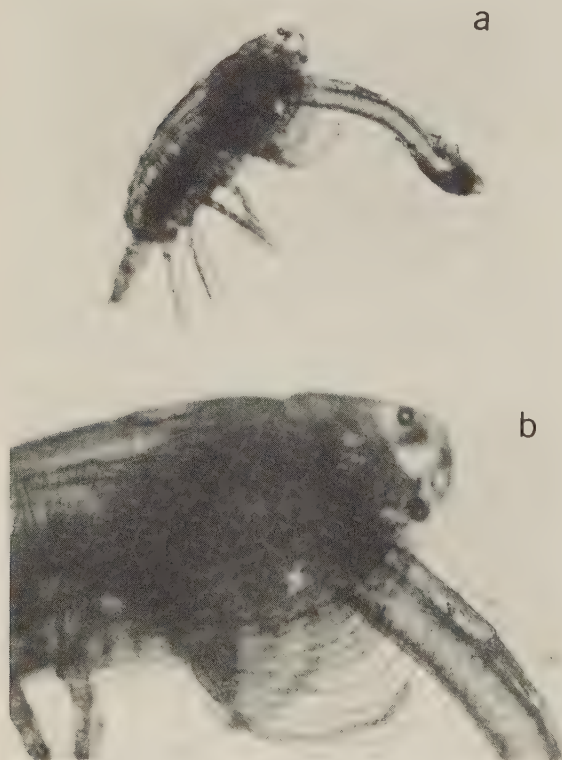


FIGURE 2.—(a) *Labidocera jollae* female, 3 mm long, and a 3-day-old anchovy larva 6 mm long which was captured by the tail and half ingested. (b) Enlargement of the head and setae of the copepod shown in 2a.

POSITIVE PHOTOTAXIS AND SWIMMING SPEED

Labidocera jollae and *L. trispinosa* are positively phototactic, and when confined to a beaker of seawater illuminated from above, concentrate at the water surface-beaker interface which is the brightest area. Copepods were induced to swim in the main body of water by wrapping the beaker with black paper with 1 cm lapped around the rim. This effectively eliminated the bright area and resulted in random swimming movements of the copepods near the surface.

Swimming distances of copepods were traced for 3 min in two dimensions on a clear acetate sheet laid over a glass plate on top of a 3.5-liter beaker. The distances were measured with a map measurer. Vertical movements were very slight, thus negligible, in these experiments because of the highly phototactic behavior of the individuals. Labidocerans can swim continually over relatively large areas in short periods of time (Vlymen, 1970). Comparative speeds for individuals are shown in Figure 4; on the aver-

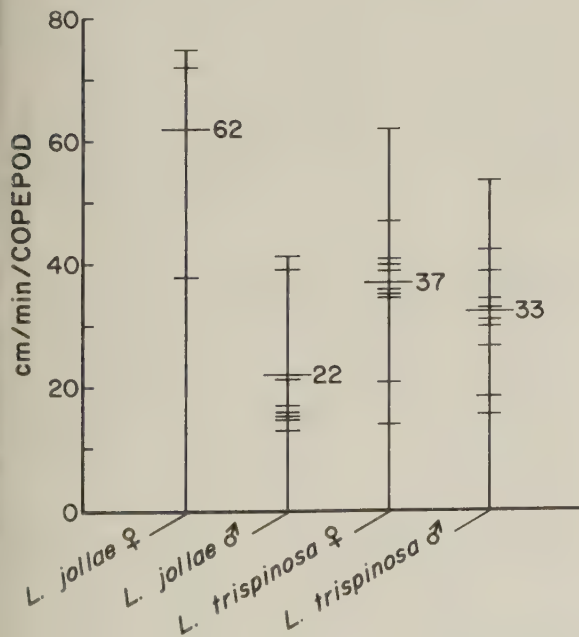


FIGURE 4.—Swimming speeds for individuals of *Labidocera*. Each small horizontal bar represents the speed of one animal; the large bar is the mean speed.

age a *L. jollae* female swims 62 cm/min (3-4 body lengths/sec) and the male swims 22 cm/min (1.5-2 body lengths/sec). Both sexes of *L. trispinosa* swim 33 to 37 cm/min (2-3 body lengths/sec). Although *L. jollae* females swim in a seemingly random pattern, the males usually swim in straight lines for a few seconds then swim in circles and cover a small area intensively.

KILLING EFFICIENCY OF *Labidocera*

We discovered that if the ratio of anchovy larvae to *L. jollae* females was low ($<10:1$), all or almost all the larvae in 3500 ml would be killed within 20 to 24 hr in the dark. Two experiments were done which illustrate this. In the first, 30 anchovy larvae were confined with a variable number of *L. jollae* females (Figure 5) resulting in concentrations of larvae to copepods

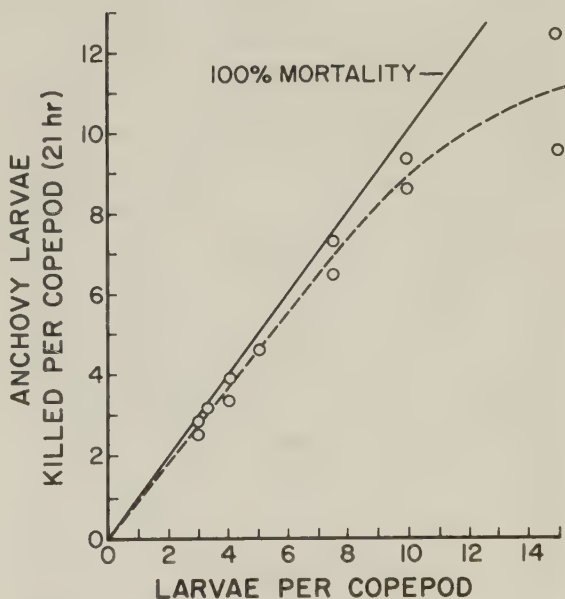


FIGURE 5.—Mortality of *Engraulis mordax* larvae, 0 to 1 day old, resulting from predation by different numbers of *Labidocera jollae* females. In each experiment 30 larvae were presented to 2 or more copepods in 3500 ml for 21 hr. Thus where 3 larvae per copepod is indicated on the abscissa, 30 larvae and 10 copepods were used; at the other extreme 15 larvae per copepod indicates 30 larvae and 2 copepods. The unbroken line is the theoretical 100% larval mortality curve.

of 3:1 to 15:1. In the other, only single *L. jollae* females were tested and the number of larvae varied to provide ratios of larvae to copepods of 5:1 to 40:1 (Figure 6). The results were

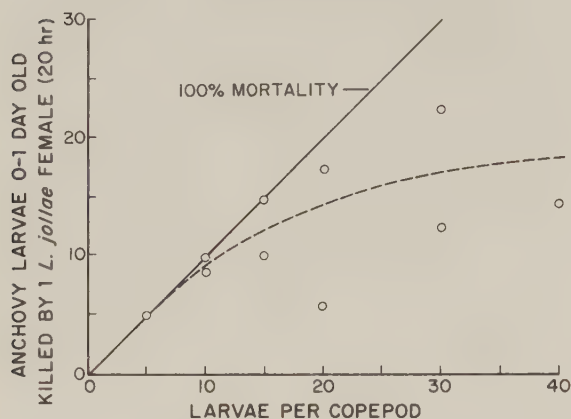


FIGURE 6.—Mortality of *Engraulis mordax* larvae, 0 to 1 day old, resulting from predation by single *Labidocera jollae* females. In each experiment 1 copepod was confined with 5 to 40 larvae in 3500 ml for 20 hr. The unbroken line is the theoretical 100% larval mortality curve.

similar in the two experiments; when the ratio was approximately 10:1 or less, it was usual for all larvae to be killed. When more than 10 larvae were available per copepod, more larvae were killed per copepod but the mortality dropped below 100%. In similar experiments two *L. trispinosa* females were tested with larvae to copepod ratios varying from 2:1 to 15:1. *L. trispinosa* females were much less efficient than *L. jollae* females and never killed all the larvae presented to them in 24 hr (Figure 7). Males of both species were similar in killing efficiency to *L. trispinosa* females. Based on these results, further predation experiments were performed over 20 to 24 hr using 30 larvae with two *L. jollae* females or five males; experiments performed with either sex of *L. trispinosa* had 30 larvae and 5 copepods. The comparative predatory ability of labidocerans is shown graphically in Figure 8. The mean number of anchovy larvae killed by *L. jollae* females was 15. *L. trispinosa* males and females had mean kills of 4 and 2

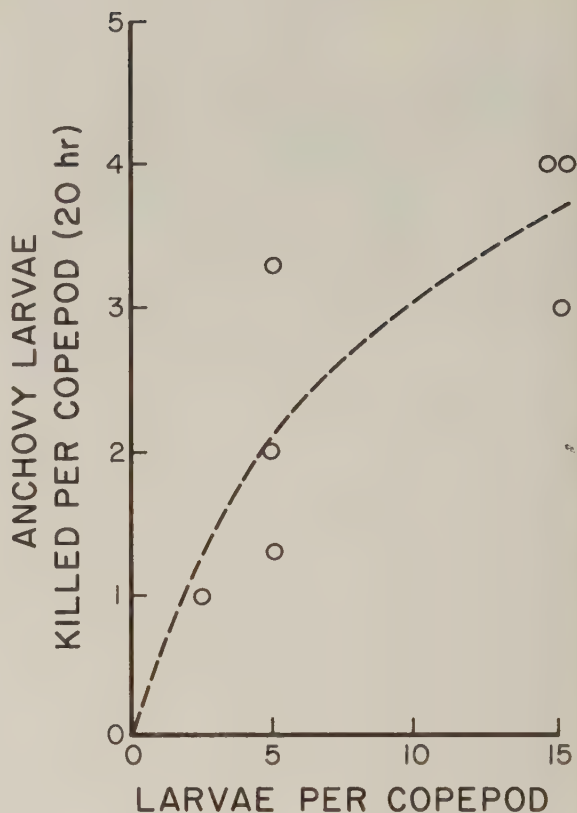


FIGURE 7.—The effect of increasing the density of larval anchovies on the predatory behavior of *Labidocera trispinosa* females. Each dot represents a separate experiment with 2 or 3 copepods and from 5 to 45 larvae.

larvae respectively in 24 hr, markedly less than *L. jollae* females. The high killing rate by *L. jollae* females reflects the longer distances and greater volume covered by them owing to their larger size. *L. jollae* females are approximately 0.2 mg dry weight, and males, 0.1 mg. Female *L. trispinosa* average about 0.1 mg dry weight; males, 0.09 mg.

EFFECT OF THE AGE OF THE LARVA ON PREDATION BY *Labidocera*

We noted in our experiments that *Labidocera* became less efficient in killing anchovy larvae as the larvae aged. The anchovy larva is 2.5 mm

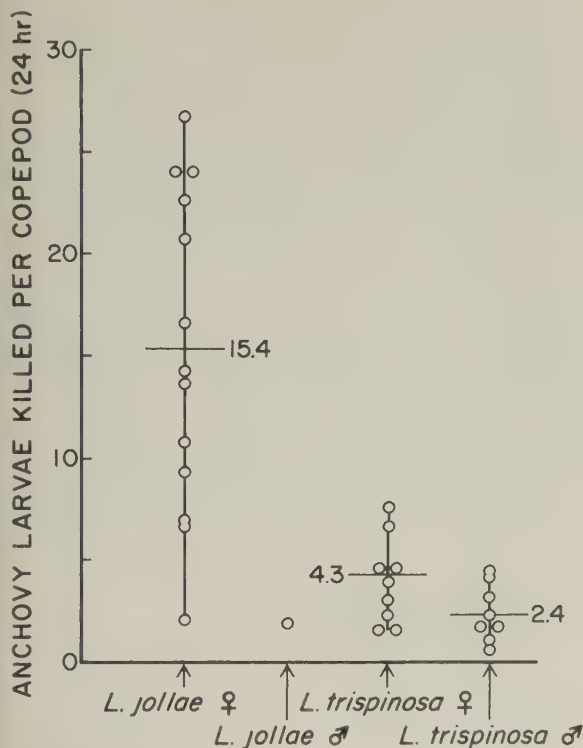


FIGURE 8.—Predation by individual *Labidocera jollae* and *L. trispinosa* under comparable prey density. Experiments were conducted in the dark for 24 hr in 3500 ml. Each circle represents the results of one experiment. The horizontal bar is the mean value for each series of experiments.

long when it hatches from the egg. Yolk-sac anchovy larvae have unpigmented eyes, lack a mouth and gills, and the yolk is invested with lipid (Bolin, 1936); when newly hatched the anchovy larva is very slightly buoyant. The newly hatched larva remains motionless most of the time at this stage and swims only sporadically. As it develops, swimming activity increases, occurring about 5% of the time at hatching to 25% on the second day and 50% by the third (John R. Hunter, personal communication). Figure 9 indicates a rapid decline in predation in the dark by *L. trispinosa* and *L. jollae* females as the larva grows older, presumably as a result of the latter's increased swimming and sensory ability. Effective predation is restricted therefore to yolk-sac larvae.

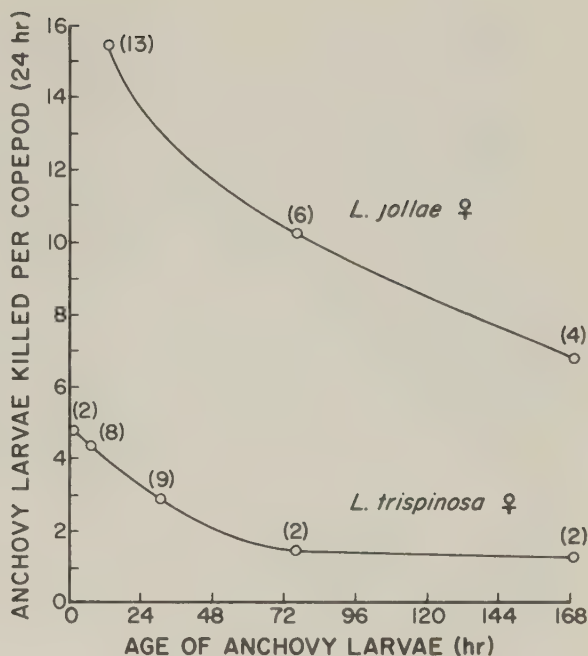


FIGURE 9.—The effect of the age of the anchovy larva on predation by *Labidocera jollae* and *L. trispinosa* females. Each open circle is the mean of the number of experiments shown in parentheses. The age of the larva at the beginning of each experiment is given on the abscissa.

EFFECT OF LARVAL ANCHOVY DENSITY ON *L. jollae* PREDATION

Given 2 to 3 days, single *L. jollae* females can kill by capture or biting all of 30 young anchovy larvae in 3500 ml. This is shown in a mortality curve (Figure 10) constructed from the results of a series of experiments, each of which had a number of newly hatched larvae (30 or less) at the start confined with a single *L. jollae* female. The density of larvae per unit volume (within the limits of these experiments) seemed to have little or no effect on the kill rate until there was only one larva remaining per 700 ml, when the rate due to predation by the copepod declined drastically. Our experience with predation experiments in 3500 ml volumes suggested that anchovy larvae were randomly distributed in this relatively small volume and that in the dark, at least, each *L. jollae* female could almost

THE EFFECT OF AN ADDITIONAL PREY ON LARVAL PREDATION BY *Labidocera*

Recent experiments by Brooks (1970) with *Labidocera trispinosa* showed that this copepod selects *Artemia salina* nauplii over copepod nauplii from the plankton. She concluded that *Artemia* nauplii are selectively grazed because they are relatively less mobile, hence more easily captured.

We tested predation by *L. trispinosa* on *Artemia* nauplii and found that grazing corresponded roughly to the results we obtained when fish larvae alone were killed, i.e., up to a certain concentration all *Artemia* nauplii were killed in the experimental container in the dark over 24 hr. Survivors were found only if the number of nauplii exceeded 11-14 nauplii/liter/copepod. As the density of nauplii was increased more were killed. This result was the same whether experiments were performed in 3500-ml beakers or in 200-ml Petri dishes (Figure 11).

When *Artemia* nauplii in various concentrations and 30 anchovy larvae were offered together to five *L. jollae* males or five *L. trispinosa* males or females, larval mortality decreased in

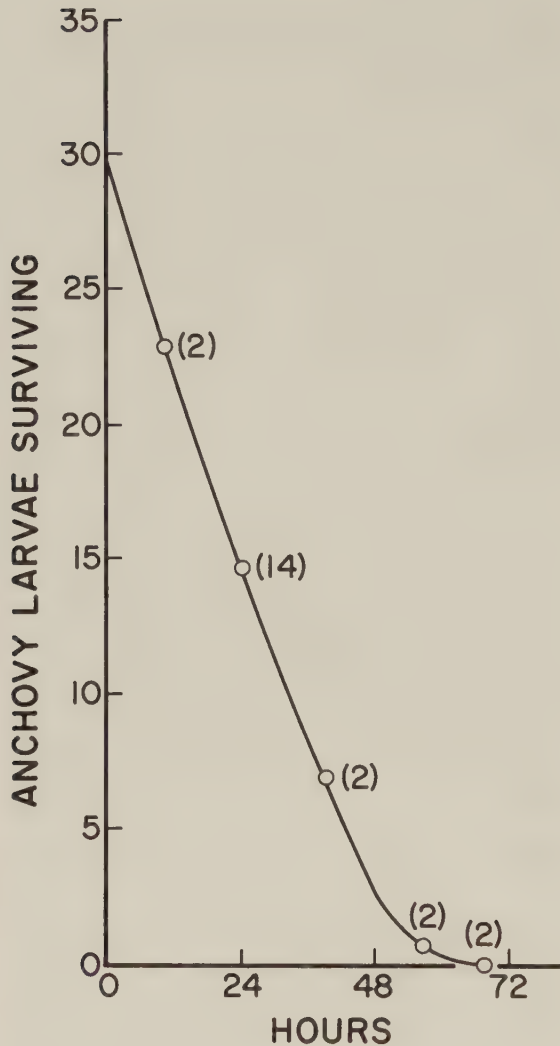


FIGURE 10.—Survival of 0- to 1-day-old anchovy larvae in the presence of a single *Labidocera jollae* female. The curve is a composite from a series of experiments in 3500 ml starting at different larval densities per copepod where the time was noted after the capture of a number of larvae. The numbers in parentheses indicate the number of experiments ending at each point.

completely search half this volume in 24 hr, resulting in continuing random contact with and killing of most, although not all, of the suspended larvae when two or more *L. jollae* females were present.

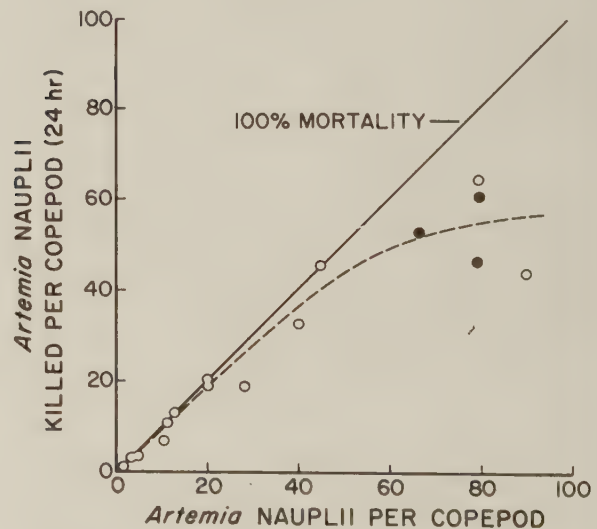


FIGURE 11.—Mortality of *Artemia* nauplii due to *Labidocera trispinosa* female predation at different densities of nauplii per copepod. Closed circles indicate experiments done in 200-ml Petri dishes.

proportion to the number of *Artemia* nauplii present. The results of these experiments are shown in Figure 12. There was a 50% decrease in mortality of larvae with *L. trispinosa* females, *L. jollae* males, and *L. trispinosa* males when the nauplii concentration was approximately 220, 150, and 100 nauplii/liter, respectively. The decrease in larval mortality is accentuated if *Artemia* nauplii are offered to *Labidocera* when older larvae are present. Fewer *Artemia* nauplii need to be present to depress the predation mortality on older larvae (Figure 13). The ease with which *Labidocera* can capture *Artemia* may make it less likely that fish larvae will be attacked. As the larvae age, this effect is compounded since it has become even more difficult to catch larvae and hence relatively less taxing for the copepod to take *Artemia* nauplii.

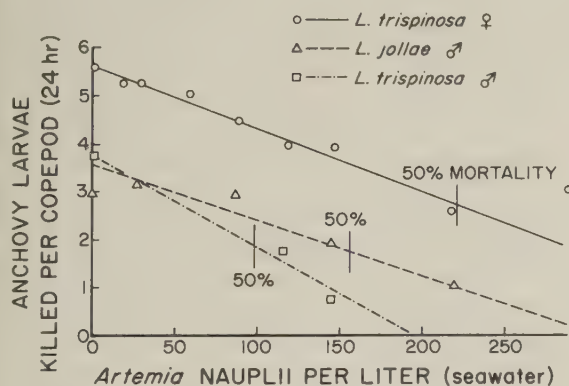


FIGURE 12.—Predation by *Labidocera jollae* males and both sexes of *L. trispinosa* on anchovy larvae when *Artemia* nauplii were also available to the copepods. Fifty percent reduction in larval mortality occurred when *Artemia* nauplii numbered 220/liter with *L. trispinosa* females, approximately 150/liter with *L. jollae* males and 100/liter with *L. trispinosa* males.

CALORIC REQUIREMENT OF *L. jollae* AND *L. trispinosa*

We noted that the vigor of *Labidocera* individuals declined with time if they were not fed or fed only *Artemia* nauplii. For example, after 1 week in the laboratory the activity of copepods fed only *Artemia* was diminished so that a copepod's ability to capture larvae was about one-

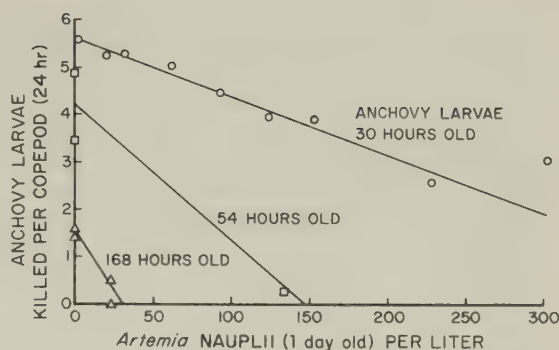


FIGURE 13.—The effect of the age of the anchovy larva and the addition of an extra prey (*Artemia* nauplii) on larval mortality due to predation by *Labidocera trispinosa* females.

half that of a newly caught copepod. *Labidocera* died after 2 or 3 days of starvation. This was preceded by a decrease in swimming activity which was reflected in a lower respiration rate and dry weight of individuals. In Figure 14 we give comparative respiration rates and dry weights for *L. jollae* females (a) newly caught, (b) larval-fish fed, and (c) starved for 2 days. These results show an enhanced respiratory rate for fed and presumably healthier animals and a drastic decline due to short term starvation.

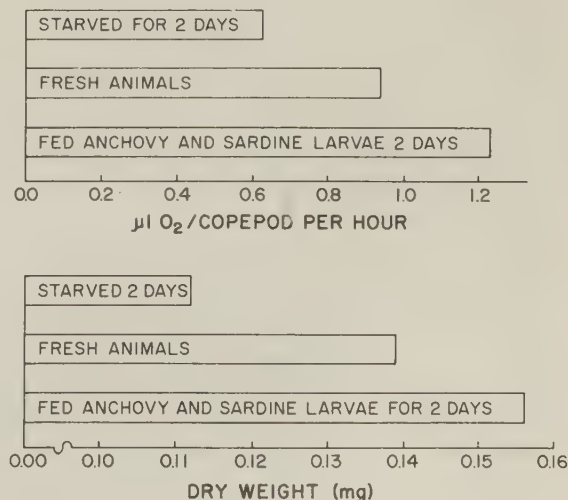


FIGURE 14.—Comparative oxygen consumption and individual dry weight measurements of *Labidocera jollae* females under starved and well-fed conditions at 18° C.

Further comparisons of respiration rates were made only between newly caught and larval-fish fed *L. jollae* females, males, and *L. trispinosa* males and females (Figure 15). In each instance there was an increase in respiratory rate after feeding on anchovy larvae. The caloric requirement of *Labidocera jollae* and *L. trispinosa* was calculated from oxygen con-

sumption data to estimate the number of larvae which could sustain the copepod in a healthy condition at 18° C (Table 1). In spite of *Labidocera's* ability to kill large numbers of fish larvae or *Artemia* in a day, actual caloric requirements may be met by ingestion of only a few (1-4) larvae or nauplii (4-16).

PREDATION ON FISH LARVAE AND *Labidocera* BY *Pontellopsis occidentalis*

Pontellopsis adults and copepodites stages IV and V can kill by biting or capture and ingestion of anchovy larvae; older larvae (up to 3.5 days old in our test) were killed by this copepod as easily as yolk-sac larvae. Each stage V copepodite killed three larvae per day on the average and each adult female killed about 11 per day (Figure 16). We observed also that *Pontellopsis* attacked and ate *Labidocera* spp. when they were confined to the same beaker.

CO-OCCURRENCES OF PREDATORY COPEPODS AND FISH LARVAE IN THE SEA

In the laboratory we noted that 30 to 40% of the *Labidocera* individuals resided in the upper 5 to 25 cm of 140-cm-deep, 17.5-cm-diameter tanks—both in darkness and in the light. Yolk-sac anchovy larvae occupied a similar stratum because they are slightly buoyant. In the sea, spawning by anchovies occurs mostly in the upper 10 m but occasionally may occur relatively deeply (Ahlstrom, 1959). This prompted us to

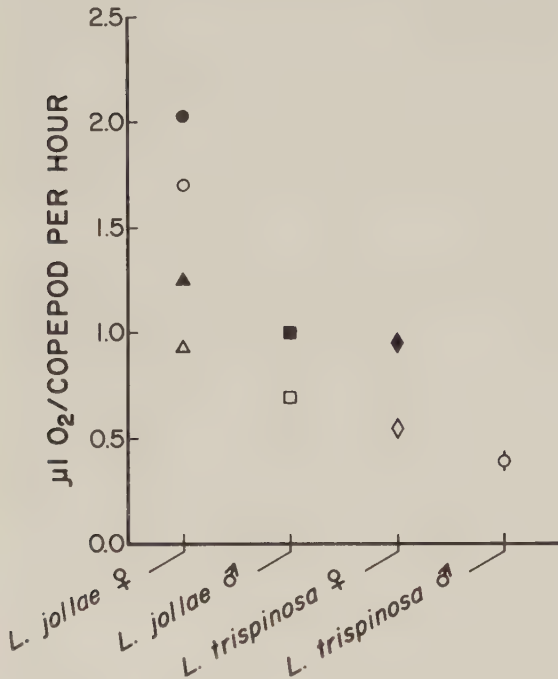


FIGURE 15.—Oxygen consumption by newly caught (open symbols) and larval-fish fed (closed symbols) labidocerae at 18° C.

TABLE 1.—Oxygen consumption and the calculated number of anchovy larvae required to sustain the respiratory requirements of *Labidocera jollae* and *L. trispinosa* per day at 18° C. The oxy-caloric equivalent of 1 μ liter of oxygen is 0.005 calorie. Yolk-sac anchovy larvae weigh 0.01 mg dry weight and contain 0.054 calorie. These data assume 100% digestive assimilation and an $RQ = 0.8$ for each copepod. Approximately four *Artemia* nauplii are calorimetrically equivalent to one anchovy larva.

Species and sex	$\dot{V} O_2$ μ liter/mg dry weight/hr	O_2 consumption μ liter/copepod/hr	Average dry weight per copepod (mg)	Anchovy larvae required/day
<i>Labidocera jollae</i> ♀	11	2.0	0.19	4
<i>L. jollae</i> ♂	11	1.0	0.095	2
<i>L. trispinosa</i> ♀	7.7	0.96	0.12	2
<i>L. trispinosa</i> ♂	4.3	0.40	0.092	1

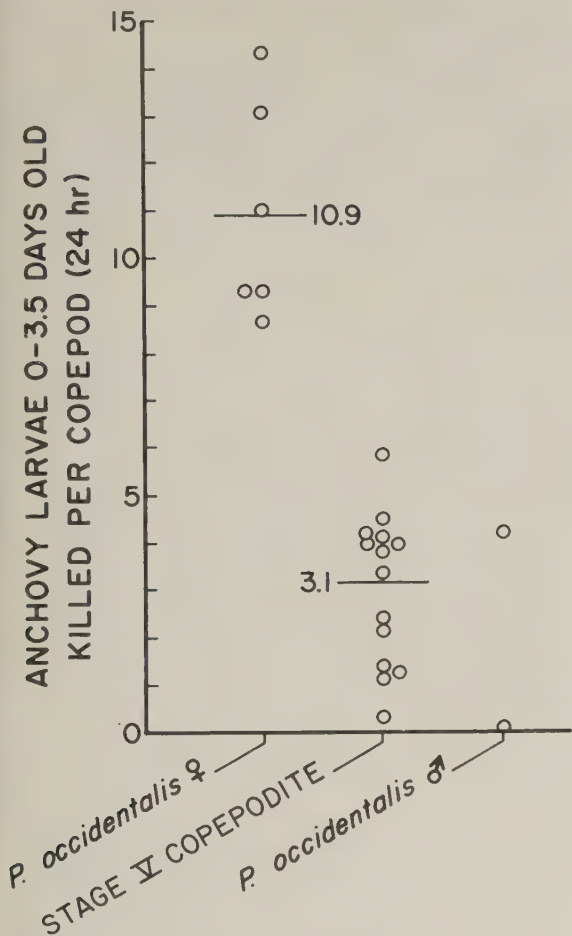


FIGURE 16.—Predation by *Pontellopsis occidentalis* on anchovy larvae 0 to 3.5 days old. Each horizontal line indicates the mean value of the experiments shown with open circles; 30 larvae were provided to 1 to 6 copepods in 3500 ml at the beginning of each experiment.

measure the rate of ascent of anchovy eggs to determine the maximum depth at which spawning could occur and yet insure the presence of yolk-sac larvae at the surface. In La Jolla seawater, salinity 33‰ and 17° C, anchovy eggs rise 5 cm/min or 3 m/hr. Thus, with time from spawning to hatching at 2 days, eggs spawned as deep as 144 m would hatch at or near the surface of the ocean, although spawning that deeply is rare (Ahlstrom, 1959). During development and, as they use up their yolk, an-

chovy larvae become almost neutrally buoyant and start to sink very slowly in laboratory containers. Even so, after 2 days of development, 50% of the laboratory-reared larvae were still above the 30 to 40 cm depth.

Ahlstrom (1959) reported closing-net captures of anchovy larvae at a variety of stations and depths in the California Current off California and Baja California. He has kindly provided us with length distributions of anchovy larvae taken at two stations, a night station, 5206-90.28, where over 500 larvae were taken, and a day station, 5504-120.50, where over 5000 larvae were captured. The length of the larva is roughly indicative of its age (Kramer and Zweifel, 1971), and we have tabulated the depth distribution of anchovy larvae at these stations by length and age (Table 2).

The depth distributions by age and length of anchovy larvae indicate that 50% or more of anchovy larvae up to 3 weeks old are above 10 m in depth. Fifty percent of the youngest class, 3 to 4.5 mm and 1 week old or less, were above 3.5 m during the day, and were slightly more than 2 m deep in the night. Ahlstrom's data also suggest that larvae of the Pacific sardine, *Sardinops caerulea*, Pacific mackerel, *Scomber japonicus*, and jack mackerel, *Trachurus symmetricus*, all pelagic fish of the California Current, may be similarly distributed.

Labidocerans are not diurnal vertical migrants and seem to be confined near the surface of the sea. Oblique tows with a plankton recorder (Longhurst et al., 1966) were taken in an area where *Labidocera trispinosa* and anchovy larvae are known to occur. The results are shown in our Table 3. The volume of each discrete sample at a particular depth was small (4-6 m³) and the zeros may simply indicate relatively low abundance below the surface. Both tows, taken a day apart in close proximity to one another, showed that *L. trispinosa* was mainly present above 10 m, as were anchovy larvae. Simultaneously 10-min neuston tows were taken which filtered 463 m³ no deeper than 30 cm at the surface of the sea (Table 4). The large number of *Labidocera trispinosa* and anchovy larvae in these tows suggests that the upper 30 cm of the ocean

TABLE 2.—Depth distribution of anchovy larvae by age and length at two stations, 5504-120.50 (day) and 5206-90.28 (night); collected by Ahlstrom (1959).

Average depth of catch (m)	Length and age of larvae							
	3.0-4.5 mm 0-7 days old		5.0-7.5 mm 8-14 days old		8.0-11.0 mm 15-21 days old		11.5+ mm 21+ days old	
	no.	% of total	no.	% of total	no.	% of total	no.	% of total
Station 5504-120.50 (day)								
2	515	42	505	17	283	37	64	36
7	317	26	820	28	216	28	52	29
18	138	11	596	20	78	10	31	17
27	96	8	138	5	31	4	5	3
44	105	9	509	17	62	8	19	11
60	61	5	324	11	95	12	7	4
74	1	0	70	2	1	0	0	0
Total	1233		2962		766		178	
Station 5206-90.28 (night)								
2	65	50	52	40	44	20	35	36
7	54	42	48	37	73	34	25	26
17	10	8	15	12	50	23	14	14
27	0	0	15	12	49	23	23	24
Total	129		130		216		97	

TABLE 3.—Vertical distribution of *Labidocera trispinosa* adults and anchovy larvae at 32°55.1' (Station 1 at 0100) June 3, 1970, and 32°45.8' (Station 2 at 2340) June 3. Volume of water filtered for each discrete depth sample in oblique tows were: Station 1, 6 m³; Station 2, 4.35 m³. No *L. jollae* or *Pontellopsis occidentalis* individuals were caught in these samples.

Depth (m)	Number of <i>L. trispinosa</i> adults	Number of <i>Engraulis mordax</i> larvae
Station 1		
0-3	13	5 (7-11 mm long)
3-7	0	1
7-15	0	1
15-60	0	0
Station 2		
0-2	4	17 (7-10 mm long)
2-7	17	11 (9-10 mm long)
7-15	2	1
15-25	0	1
25-60	0	0

is the area which should be examined for further elucidation of this predator-prey relationship.

The observations presented in this paper indicate that marine copepods may be effective predators on larval fish, at least in the sense that a predator need not devour its prey but is equally effective if it injures it mortally. Young pelagic fish larvae are particularly susceptible to biting zooplankters because they have an extremely thin skin and are unable to survive once the skin is punctured. Pontellid copepods, in particular *Labidocera* spp., appear to have a well-developed

TABLE 4.—Numbers of *Labidocera jollae*, *L. trispinosa*, *Pontellopsis occidentalis*, and *Engraulis mordax* eggs and larvae taken in 463 m³ within 30 cm of the surface simultaneously with the oblique tows described in Table 3.

Species	Station 1	Station 2
<i>L. trispinosa</i>	1152	6400
<i>L. jollae</i>	336	104
<i>P. occidentalis</i>	112	128
<i>E. mordax</i>	226	36
	(5-10 mm long)	(10-12 mm long)

vibration sense which serves to orient the copepod toward its swimming prey although this is preceded by random searching. A fish larva with its beating tail provides the right stimulus to the copepod to initiate an attack when the latter is close enough to detect the beat.

We have called attention to the vertical distribution of *Labidocera* in the sea and the apparent co-occurrence of larval anchovies in the same depth stratum. Unfortunately, quantitative data on the density of predatory copepods or other zooplankters as related to fish larvae have yet to be made. It is our opinion that pontellid copepods and fish larvae are concentrated in the upper few meters and probably the upper few centimeters of the sea and that observations of this oceanic fine structure may reveal densities of fish larvae to copepods which would implicate predatory copepods (and possibly other zooplankters) as important causes of larval fish mortality.

ACKNOWLEDGMENTS

We thank James E. Smith and Donald L. Seibert of the Scripps Institution of Oceanography and Michael McMaster, Mrs. Gail Theilacker, and Raymond E. Shuey of the National Marine Fisheries Service, Fishery-Oceanography Center, for their generous assistance during this study.

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TROPHIC INTERACTION BETWEEN THE SEA STAR *Pisaster giganteus* AND THE GASTROPOD *Kelletia kelletii*

RICHARD J. ROSENTHAL¹

ABSTRACT

The sea star *Pisaster giganteus* and the gastropod *Kelletia kelletii* are conspicuous inhabitants of the sublittoral zone off San Diego, Calif. Diving observations over a period of 2½ years indicate that the two species are trophically interrelated. *P. giganteus*, an opportunistic predator, and *K. kelletii*, a carnivorous scavenger, have been observed feeding together on common food items. The sea star appears to be a major predator of the whelk, even though *K. kelletii* made up less than 10% of the diet of the sea star. The whelk does not display an avoidance response in the presence of *P. giganteus*. Coexistence between the two species is believed possible as long as *K. kelletii* does not become more preferred prey of the asteroid.

Available information on the behavioral responses of marine mollusks in the presence of predatory sea stars has increased markedly within the past few decades (Bullock, 1953; Feder, 1963, 1967; Margolin, 1964a, 1964b; Feder and Christensen, 1966; Montgomery, 1967). However, most of these investigations have been limited to laboratory or intertidal observations. Except for a recent study by Mauzey, Birkeland, and Dayton (1968), the interactions between mollusks and sea stars in the eastern Pacific subtidal waters have not been investigated. Direct sublittoral behavioral observations off the west coast of north America have been hampered by cold water and limited underwater observation time.

Assessment of predator-prey relationships between subtidal organisms has been limited mainly to recording interactions between two organisms under laboratory conditions. A species-specific avoidance reaction or escape response by a mollusk to a sea star is considered one indication of a predator-prey relationship. The evolution of such responses, and the recognition of chemical stimuli emanating from either

organism, suggests a long standing predator-prey association. Mauzey et al. (1968) found, however, that biochemical similarities between a predator and other organisms could cause a prey species to avoid the nonpredatory species as well as the predator.

Gastropods which displayed no avoidance responses in the presence of specific sea stars have been observed by Bullock (1953), Margolin (1964b), and Feder (1967). Bullock (1953) even suggested that nonresponsive mollusks are characteristic of ecological situations where starfish predation on these species must be rare.

This paper examines laboratory and field data obtained on the behavioral interactions between the sea star *Pisaster giganteus* (Stimpson) and the gastropod *Kelletia kelletii* (Forbes). Included are observations on the feeding, species-specific responses, and predator-prey interaction between the two species.

P. giganteus is reported from Vancouver Island, British Columbia, to northern Baja California, Mexico (Fisher, 1930), while *K. kelletii* has been found from Santa Barbara, Calif., to San Quintin Bay, Baja California, Mexico (Abbott, 1954). Both species are conspicuous and abundant inhabitants of the nearshore subtidal reefs off southern California. Bathymetric distribution appeared to be somewhat similar for *K. kelletii* and *P. giganteus* off San Diego County,

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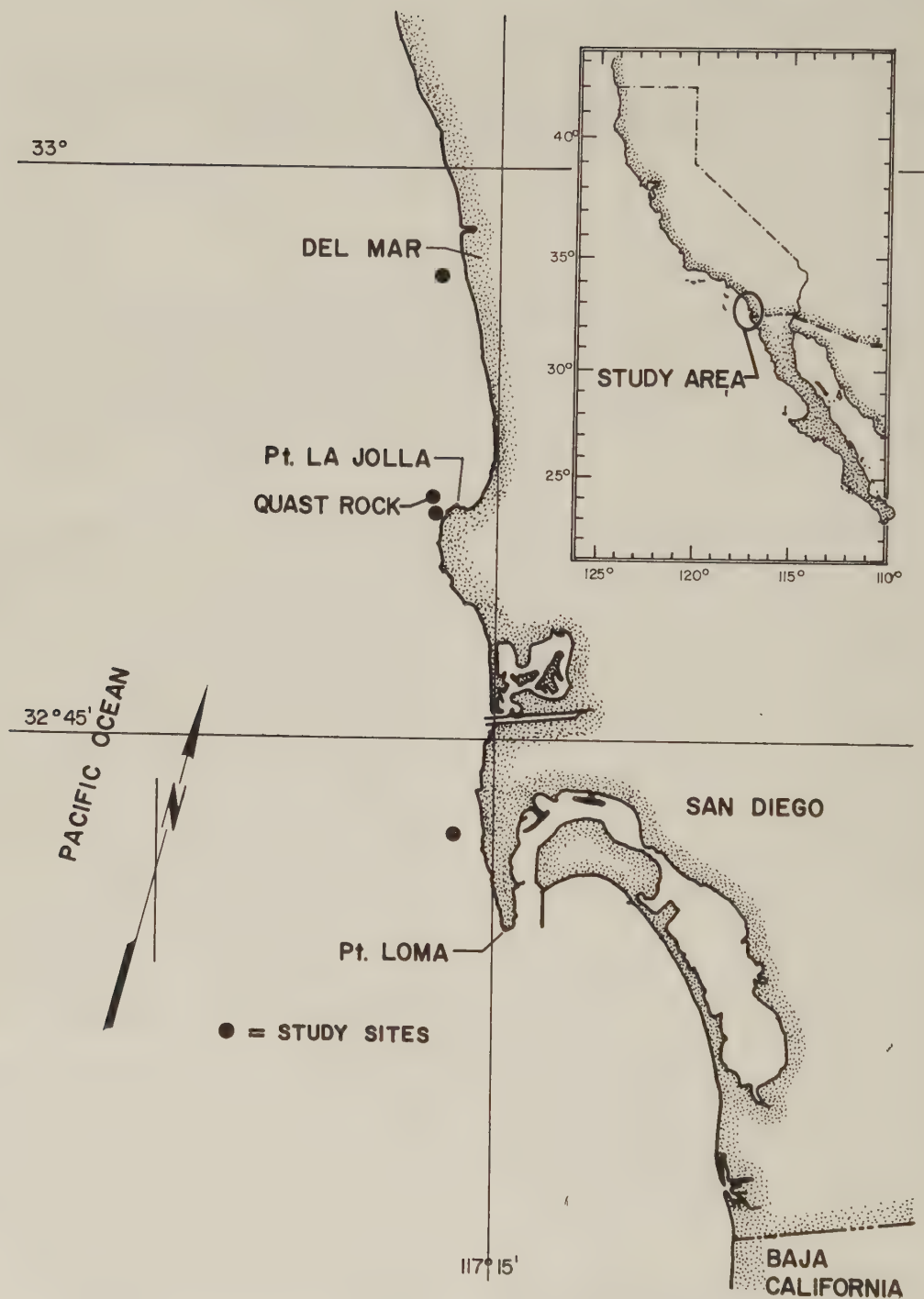


FIGURE 1.—Location of the four subtidal observation areas off San Diego County, Calif.

with the greatest concentrations of each between 2 and 40 m in depth.

The laboratory portion of the study was conducted in the experimental seawater aquarium of the National Marine Fisheries Service, Fishery-Oceanography Center, La Jolla, Calif. I made direct subtidal observations during daylight hours while scuba diving off San Diego County during the period January 1968-July 1970.

DESCRIPTION OF SUBTIDAL STUDY AREAS

Four widely separated nearshore locations within San Diego County were selected as field study sites (Figure 1). These sites were selected because they varied in depth, substratum, and species composition.

DEL MAR

The study area was located within a stand of giant kelp, *Macrocystis pyrifera*, which lies in 15 to 20 m of water about 1 km offshore from Del Mar, Calif. (lat 32°57' N, long 117°16' W). The kelp bed is characterized by having a relatively flat sea bottom with intermittent sand patches and low profile siltstone ledges. The sea floor is relatively homogeneous in appearance except for the occurrence of these ledges, which are less than 1.5 m in height. *Pterygophora californica*, a low standing brown algae, occurs abundantly on the seaward edge of the *M. pyrifera* bed.

POINT LA JOLLA

The observation site off Point La Jolla (lat 32°51' N, long 117°16'30" W) was between 150 and 300 m due west of Point La Jolla. The area is characterized by large boulders and undercut sandstone ledges. It is a topographically heterogeneous substrate containing many microhabitats. Portions of the area contain large sandstone formations which rise vertically to within a few meters of the sea surface. The tops of

these formations are often covered by surf grass, *Phyllospadix torreyi*. The observation area ranged between 5 and 16 m deep because of such pronounced vertical changes in relief. Scattered throughout the area are two species of perennial brown algae, *Egregia laevigata* and *Eisenia arborea*.

QUAST ROCK

Quast Rock is situated on an offshore reef about 630 m northeast of Point La Jolla (lat 32°51'30" N; long 117°17' W). The observation area encompassed approximately 225 m² of this reef. The rock is a sandstone formation with a deep undercut and a cave on the northern end. The substratum supports an extremely diverse benthic invertebrate fauna, largely because of the complexity of the habitat. The area is devoid of giant kelp and only two species of brown algae, *Cystoseira osmundacea* and *Agarum fimbriatum*, were common along the reef. The observation area ranged from 17 m on the top of the rock to approximately 27 m on a lower terrace.

POINT LOMA

The study site was located approximately 1.5 km offshore from Point Loma, Calif. (lat 32°42' N; long 117°16' W). The area is within a *M. pyrifera* stand and the bottom is between 13 and 18 m deep. The substratum is predominantly rock, siltstone, and sand. Portions of the bottom are interrupted by channels and low relief ledges. The shade area under the giant kelp canopy supports an algal undergrowth composed primarily of *P. californica*, *C. osmundacea*, and *Laminaria farlowii*.

FEEDING BEHAVIOR OF *Pisaster giganteus*

P. giganteus appears to be an opportunistic predator on the nearshore subtidal reefs off San Diego County. It feeds primarily upon live animals, although it has been observed scavenging on dead fishes and invertebrates. Thirty-two identifiable species of invertebrates, with pelecypods and gastropods making up about 80%

of the total, were seen being preyed on by this sea star at the four study areas (Table 1). The diet of *P. giganteus* is remarkably variable or generalized over the four subtidal observation areas, but within each habitat the diet is more

TABLE 1.—A list of prey species eaten by *Pisaster giganteus* and *Kelletia kelletii* from January 1968 to July 1970.

Prey	(Class)	<i>Pisaster giganteus</i>	<i>Kelletia kelletii</i>
<i>Anomia peruviana</i> (pelecypod)		+	
<i>Astraea gibberosa</i> (gastropod)		+	
<i>Astraea undosa</i> (gastropod)		+	+
<i>Balanus tintinnabulum</i> (crustacean)		+	+
<i>Botula (Adula) falcata</i> (pelecypod)		+	+
<i>Bursa californica</i> (gastropod)			+
<i>Ceratostoma nuttallii</i> (gastropod)		+	
<i>Chama pellucida</i> (pelecypod)		+	+
<i>Conus californicus</i> (gastropod)		+	+
<i>Diopatra ornata</i> (polychaete)		+	+
<i>Hinnites multirugosus</i> (pelecypod)		+	+
<i>Jaton festivus</i> (gastropod)		+	+
<i>Kelletia kelletii</i> (gastropod)		+	+
<i>Lithophaga plumula</i> (pelecypod)		+	+
<i>Loligo opalescens</i> (cephalopod)		+	+
<i>Maxwellia gemma</i> (gastropod)		+	
<i>Mitra idae</i> (gastropod)		+	
<i>Mytilus californianus</i> (pelecypod)		+	
<i>Mytilus edulis</i> (pelecypod)		+	
<i>Netastoma rostrata</i> (pelecypod)		+	
<i>Ostrea lurida</i> (pelecypod)		+	
<i>Panulirus interruptus</i> (crustacean)		+	+
<i>Paralabrax nebulifer</i> (osteichthyes)		+	+
<i>Parapholas californica</i> (pelecypod)		+	+
<i>Pelagia panopyra</i> (scyphozoon)		+	
<i>Phyllochaetopterus prolifica</i> (polychaete)		+	+
<i>Pisaster giganteus</i> (asteroid)			+
<i>Platyodon cancellatus</i> (pelecypod)		+	+
<i>Pododesmus cepio</i> (pelecypod)		+	+
<i>Pterynotus trialatus</i> (gastropod)		+	
<i>Pyura haustor</i> (ascidian)		+	
<i>Serpulorbis squamigerus</i> (gastropod)		+	
<i>Sphyræna argentea</i> (osteichthyes)		+	+
<i>Strongylocentrotus franciscanus</i> (echinoid)		+	+
<i>Strongylocentrotus purpuratus</i> (echinoid)		+	+
<i>Styela montereyensis</i> (ascidian)		+	
<i>Talipes nuttallii</i> (crustacean)			+
<i>Ventricularia fordii</i> (pelecypod)		+	+

¹ (S) = Scavenge.

selective or restricted (Figure 2). Variations in the diet of *P. giganteus* with each habitat or microhabitat are attributed to the availability and abundance of prey organisms, and preferences in the feeding behavior of the sea stars in these four locations.

Predator success may, in many instances, be dependent upon the predator's ability to feed upon what is available in a given habitat. The prey must be abundant enough to be utilized as a food source and the predator must be capable of selecting these forms. Variability in diet

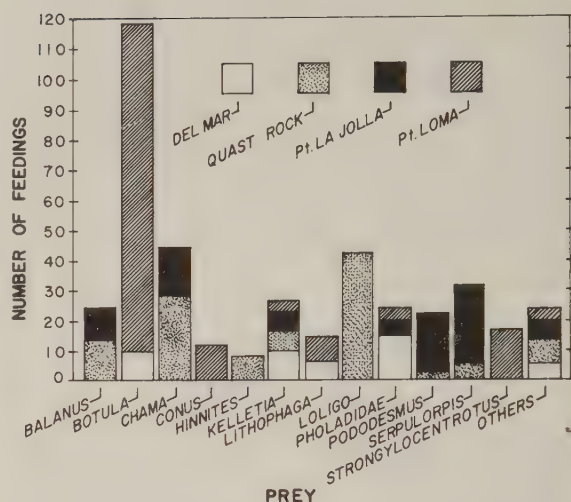


FIGURE 2.—Comparison of the feeding behavior of *Pisaster giganteus* as observed in the four study sites from August 1969 to July 1970.

with changes in sea star habitat has been discussed by Mauzey et al. (1968). Feder (1959) suggested that differences in the diet of the sea star *P. ochraceus* can largely be attributed to changes in prey availability.

Evidence for opportunistic or adaptable predation by *P. giganteus* is drawn from variability in diet within each habitat and a temporary alteration in the feeding behavior of the Quast Rock sea star population. On February 20, 1970, 12 out of 43 *P. giganteus* observed on or around Quast Rock were found to be feeding. Six of these individuals were eating *Chama pellucida*; two, *Balanus tintinnabulum*; two, *Serpulorbis squamigerus*; and one, *Pyura haustor*. Alteration in feeding was observed with the sudden appearance of a potential food source during the spring of 1970. Thousands of spawning squid, *Loligo opalescens*, were observed in the proximity of Quast Rock on March 12, 1970. Spawning had been reported to occur each year in the La Jolla area (Fields, 1965), although "spent" individuals had not been sighted in the Quast Rock location during the previous 12 months. Numbers of dying or dead squid were lying along the bottom off Quast Rock. A total of 52 *P. giganteus* was examined around Quast Rock; of these,

42 were feeding on dead *L. opalescens*. The *P. giganteus* population had temporarily switched from active predators to scavengers with the availability of this abundant, though temporary, food source. I returned to the study site on April 10, 1970, and noted 6 out of 41 *P. giganteus* to be feeding equally on *C. pellucida*, *B. tintinnabulum*, and *Hinnites multirugosus*. With the dead squid no longer present, the sea star population returned to feeding on the available prey organisms of the reef.

Feeding preference experiments by Mauzey et al. (1968) and Landenberger (1968) indicated that the sea star *P. ochraceus* preferred mussels to alternative food items offered the sea stars. Landenberger (1968) also showed that *P. giganteus* preferred the mussels *Mytilus edulis* and *M. californianus* to five other molluscan species. Additional observations by Paine (1969) suggest that few other prey are consumed as long as mussels are readily available to *P. ochraceus*. However, mussels were rarely available to *P. giganteus* in the four subtidal locations examined. If *P. giganteus* has a specific food preference in these subtidal locations, then it must be adaptable or capable of change, since each habitat or microhabitat varies somewhat in prey availability.

P. giganteus appears to exhibit a feeding preference for prey that is either immobilized or sedentary in habit, since these organisms were eaten more frequently than motile forms. Attached or boring bivalves, balanoid barnacles, and sessile tube-dwelling mollusks were "preferred" or eaten most often by *P. giganteus*.

P. giganteus preys on *K. kelletii* more often than any other motile gastropod, and yet the whelks do not appear to be eaten in proportion to their abundance or accessibility in these locations. Abundance and distribution of *K. kelletii* was determined by random sampling along 150 m transect lines in two of the locations. The following densities were determined: Del Mar 1.82/m², and Point Loma 0.73/m². Distribution and movement of *K. kelletii* is, at least, sometimes nonrandom, for the whelks were found to be in an aggregated distribution pattern in each of these areas during late spring of 1970. The distribution pattern of a motile organism

may reflect spawning or feeding interactions; therefore, these results were compared with and found to coincide with earlier data obtained from the Del Mar kelp bed during the fall of 1968. During the reproductive season (Rosenthal, 1970), numbers of *K. kelletii* occur in communal spawning groups, and yet predation by *P. giganteus* did not appear to increase with the availability of the whelks in these locations.

FEEDING BEHAVIOR OF *Kelletia kelletii*

K. kelletii is basically a carnivorous scavenger, although it has been observed feeding on live sedentary polychaetes. As a scavenger, it appears to be attracted to almost any injured or dead animal occurring on the sea floor (Table 1). Often, large numbers of *K. kelletii* have been observed moving towards and/or feeding upon a common food item in subtidal areas. The food-finding ability of *K. kelletii* by distance chemoreception has, on more than one occasion, been a nuisance to spiny lobster fishermen in some areas off southern California. These fishermen usually bait traps with dead fish to attract the spiny lobster *Panulirus interruptus*. Many times, however, a single lobster trap may contain dozens of *K. kelletii* which were attracted to the trap by the "scent" of the bait.

K. kelletii feeds with an extensible muscular proboscis which can be extended from the head region during feeding. Food is ingested by a muscular sucking action of the proboscis and a rasping of the radula. The proboscis is capable of extending approximately twice the length of the whelk's shell; it is this extension which allows *K. kelletii* to reach food items in depressions or within the substratum. Pearce and Thorson (1967) found that the proboscis of the gastropod *Neptunea antiqua* can be everted and may be extended to 2¼ times the length of the snail's own shell.

The proportion of the whelk's diet which results from preying on live animals versus scavenging on carrion or dying organisms was not determined. An aggregation of *K. kelletii* feeding on a dead fish or mollusk attracts the attention of an underwater observer more often than



FIGURE 3.—*Pisaster giganteus* and *Kelletia kelletii* jointly feeding on a date mussel, *Botula (Adula) falcata*.
Note: The sea star's tube feet are touching the whelk's foot.

an isolate feeding. In many instances the snails were believed to be feeding since the proboscis was extended into a hole or within the substratum, and yet rarely was the food item identified.

Most of the scavenger feedings by *K. kelletii* attract more than one individual. In one instance, 85 *K. kelletii* were clustered around and feeding on a dead bass, *Paralabrax* sp., off Point Loma. Aggregate feedings on carrion or dying animals appeared to be the usual mode of feeding in these subtidal areas.

CONVERGENT FEEDING BEHAVIOR

A unique and yet reoccurring behavior pattern has been observed in those subtidal habitats where both species are found. *P. giganteus* and *K. kelletii* converge and attempt to feed on a common prey organism at the same time (Figure 3). Usually *P. giganteus* began to feed first, with the whelks grouped around the sea star (Figure 4). In many instances *K. kelletii* were



FIGURE 4.—Convergent feeding behavior between *Pisaster giganteus* and *Kelletia kelletii* 21 m underwater off Point La Jolla, Calif. The sea star is eating a *Chama pellucida*, while three whelks are attempting to get to the prey.

partially underneath or even crawling over the sea star attempting to get to the food item, and at times a feeding sea star would also be grasping from one to four *K. kelletii*. A few of these interspecific feedings were quite distinct in appearance, especially when both species were scavenging larger prey such as dead fishes. Three *P. giganteus* and 28 *K. kelletii* were observed feeding simultaneously on a dead barracuda, *Sphyræna argentea*, off Quast Rock on December 31, 1968. However, most of the feeding convergences observed were not as obvious as this; usually they involved only a single sea star and two or three whelks. Convergent feeding behavior has been noted 65 times in these four locations over a 2-year period. On one occasion, five separate convergent groups were observed within a 400 m² area off Point Loma.

Direct competition for food was most evident when both species were scavenging carrion or moribund organisms. However, in situations where *P. giganteus* had captured or killed live prey, *K. kelletii* was rarely observed with its proboscis extended into the prey. It is probable that feeding by the whelk usually takes place on a secondary basis after the sea star has departed. The percentage of prey that is left uneaten by *P. giganteus* following a feeding is unknown.

These convergent feeding groups were not limited to *K. kelletii* and *P. giganteus*. *K. kelletii* has been observed feeding interspecifically with two other sea stars, *Dermasterias imbricata* and *Pisaster brevispinus*. Other scavenging or carnivorous types of epibenthic invertebrates which have been found in these sea star-whelk feeding groups include the gastropod *Mitra idae* and the hermit crab *Paguristes ulreyi*.

PREDATION ON *Kelletia kelletii*

There have been few natural predators of *K. kelletii* reported in the literature. The moon snail, *Polinices lewisii*, was observed by MacGinitie and MacGinitie (1949) to have drilled and eaten a live *K. kelletii* in a laboratory tank. Juvenile *K. kelletii* were found in the stomachs

of young pile perch, *Rhacochilus vacca*, by Limbaugh (1955). Three predators of *K. kelletii* observed during diurnal hours in these subtidal locations were *Pisaster brevispinus*, *P. giganteus*, and the cephalopod *Octopus bimaculatus*. However, only predation by *P. giganteus* is considered at this time.

P. giganteus is a major predator of *K. kelletii* on the nearshore reefs that I studied. During the 2½-year study period, 42 separate feedings were observed in which the sea star was in the act of digesting a whelk; and 53 other times, *P. giganteus* were found attacking *K. kelletii*. Five separate attacks by *P. giganteus* on *K. kelletii* were observed in a single dive off middle Coronado Island, Baja California (lat 32°25' N, long 117°16' W) on December 3, 1969.

The sea star usually fed by attaching tube feet to the substratum and the shell and the operculum of the *K. kelletii*. *P. giganteus* was usually observed in a humped or arched position while attacking and feeding on the whelk. In this feeding position, a sea star can bring into play a greater number of tube feet and exert maximum pull on the operculum and shell of the whelk while still remaining attached to the substratum (Feder and Christensen, 1966). *K. kelletii* were not swallowed or ingested whole by *P. giganteus*; instead, digestion appeared to take place extraorally as is the case with other food items. The whelk's operculum was either torn away or pulled out of the shell opening by the tube feet, and the sea star's stomach was inserted into the shell. Bullock (1953) suggested that predation on the intertidal snail *Acanthina spirata* by carnivorous asteroids was slight, and that possibly possession of a heavy operculum accounted for the nonresponsive behavior the snail displayed in the presence of predatory sea stars. The operculum of *K. kelletii* does not eliminate sea star predation; however, the structure apparently does increase the time necessary for an asteroid to complete the feeding process.

The *K. kelletii* which were attacked by *P. giganteus* ranged between 18 and 120 mm in shell length (siphonal canal to the apex); however, approximately 70% of these feedings involved *K. kelletii* greater than 60 mm in length. This size class, greater than 60 mm, was com-

posed primarily of sexually mature individuals (Rosenthal, 1970). *K. kelletii* less than 40 mm in length were rarely preyed on by *P. giganteus*. These smaller individuals are more secretive in habit than larger mature *K. kelletii*, and they characteristically burrow in the substratum. Possibly the burrowing and cryptic behavior of smaller *K. kelletii* decreases sea star predation by reducing the number of contacts with predatory *P. giganteus*. *K. kelletii* is considered to be what Paine (1969) referred to as "secondarily preferred prey," since invertebrate species other than *K. kelletii* were found to be numerically more predominant in the diet of *P. giganteus* at these four locations (Figure 2).

Seasonal changes in *K. kelletii* predation by *P. giganteus* was not considered to be a factor in these subtidal regions. *P. giganteus* was observed feeding on whelks throughout the year with no noticeable change in the incidence of predation.

RESPONSE OF *Kelletia kelletii* TO *Pisaster giganteus*

From the number of reports of mollusks responding to predatory sea stars (Feder and Christensen, 1966), I initially expected to observe an avoidance reaction by *K. kelletii* in the presence of *P. giganteus*. To test this, 150 *K. kelletii* between 15 and 138 mm in shell length were brought into the laboratory and maintained in either standing or circulating seawater. The seawater temperature ranged between 16.0° and 19.9° C throughout the entire experiment. *K. kelletii* were tested for any reaction which might be exhibited in the presence of, or while touching, *P. giganteus*. At times, the whelks responded by siphon extension, shell rocking, or twisting, and a slow sliding movement away from or in the direction of *P. giganteus*. Many times no shell movement was noted within a 10-min period. Tests were conducted on whelks that were inactive and on others that were moving, feeding, or spawning. Each *K. kelletii* was used only once or twice so that continual contact with the sea star would not affect the whelk's reaction.

At no time did I note an escape or avoidance response by *K. kelletii* in the presence of the sea star.

Field observations were similar to those in the laboratory; either *K. kelletii* did not respond or, at times, they actually were attracted to *P. giganteus*. The two species were usually found close to one another on these subtidal reefs, and contacts between the two probably are frequent. During convergent feeding, the two species touched or even crawled over one another; however, at other times the *K. kelletii* and *P. giganteus* occurred within a few centimeters of each other even though neither species was engaged in feeding. Paine (1969) found a perplexing intimacy of association between three intertidal gastropods and their major predator, the sea star *P. ochraceus*. In contrast to these two situations, Bullock (1953: 137), stated that "In those seashore situations where predatory starfish and gastropods both occur, it is notable that the two are generally not seen close together."

One of the routine field experiments was to pick up a *P. giganteus* underwater and place it on or within a few centimeters of an individual or group of *K. kelletii*. On only one occasion was a reaction exhibited in *K. kelletii* out of the hundreds of attempts to stimulate an active response. In this one instance, a group of 11 *K. kelletii*, which appeared to be searching for food, was encountered 20 m underwater off Del Mar on November 6, 1968. A large *P. giganteus* was placed approximately 30 cm from the group of whelks, and within a few minutes the sea star approached the *K. kelletii*. All of the whelks moved away from the approaching sea star; however, two of the *K. kelletii* were captured by the sea star while the others moved off in a similar direction. This reaction appeared to be an avoidance response although it could have been only random or chance movement on the part of the whelks, regardless of the presence of the sea star.

CONCLUSION

The existence of a predator-prey relationship between a gastropod and a sea star is not unusual; however, the continual nonresponsive or

at times even attractant behavior the whelk displays in the presence of this potential predator appears to be unique. *P. giganteus* and *K. kelletii* are trophically interrelated. Trophic interaction is based on a predator-prey relationship and similarities in the diet of both species. The prey species consumed by *P. giganteus* and *K. kelletii* are similar; however, the method of feeding, as well as the physical condition of the prey, is usually dissimilar.

These observations leave many unanswered questions on the behavioral relationship between a potential prey organism and a predatory sea star. *K. kelletii* is preyed upon by *P. giganteus*; however, the whelk does not respond to sea star contact as has been reported in other gastropod-asteroid interactions. The sea star is a major predator of *K. kelletii*, although the whelk makes up less than 10% of the sea star's diet. The factors which limit predation of *K. kelletii* by *P. giganteus* are unknown, since both species are such conspicuous and extremely abundant inhabitants of the sublittoral zone off San Diego County. Feder (1963) studied intertidal sea star predation on gastropods and noted that species which exhibited avoidance responses in the presence of asteroids were not preyed upon in proportion to their abundance or availability in the intertidal. *K. kelletii* does not exhibit avoidance or escape responses in the presence of *P. giganteus*, and yet it does not appear to be eaten in proportion to its accessibility or abundance in subtidal areas off San Diego. A general feeding preference by the sea star for attached or nonmotile prey is thought to limit predation on *K. kelletii* in these locations. The cryptic and burrowing habits of juvenile *K. kelletii* may further reduce predation on the smaller whelks by limiting the number of contacts with predatory sea stars. Paine (1969) suggested that coexistence between a major predator and prey is possible as long as the prey species does not reach a more preferred status in the diet of the predator.

It can only be speculated that the escape response of *K. kelletii* in the presence of the sea star has either not evolved or possibly was lost through continual contact and convergence on a similar trophic level. Perhaps other behavioral

activities such as feeding may be of greater selective value to *K. kelletii* than is a species-specific avoidance response. Quite possibly the whelk benefits more by not actively avoiding *P. giganteus* than it would by continually running from this potential predator. The chances for *K. kelletii* to feed on moribund or dead organisms would be increased, since both species are attracted to these food items.

SUMMARY

1. Trophic interaction between the sea star *P. giganteus* and the gastropod *K. kelletii* is based on a predator-prey relationship and similarities in the diet of both species.

2. *P. giganteus*, a highly opportunistic predator, was observed feeding on 32 identifiable species of invertebrates. The sea star exhibited a preference for prey which was either immobilized or sedentary in habit, since these forms were preyed upon most heavily.

3. *K. kelletii*, a carnivorous scavenger, usually feeds on moribund or dead organisms that it finds resting on the sea floor.

4. *K. kelletii* and *P. giganteus* feed together on common food items. Convergent feeding between these two species was a recurring behavioral pattern which was observed repeatedly in the four subtidal areas.

5. *P. giganteus* is the major identifiable predator of *K. kelletii* off San Diego County; however, the whelk makes up less than 10% of the prey observed to be captured by the sea star. The whelk is believed to be of secondary importance as food of the sea star, since invertebrates other than *K. kelletii* were utilized more often.

6. *K. kelletii* did not display a species-specific avoidance or escape response in the presence of, or while in contact with, *P. giganteus*.

7. Both species appear to be highly successful and abundant organisms of the sublittoral zone, and coexistence is believed possible as long as predation by the sea star on *K. kelletii* is not excessive.

ACKNOWLEDGMENTS

I am grateful to the National Marine Fisheries Service, La Jolla, Calif., for providing laboratory facilities. This manuscript has benefited from the critical reviews of J. R. Chess, W. D. Clarke, and H. M. Feder. Thanks are expressed to R. E. Bower and J. R. Chess for the help and assistance they rendered in the field while diving.

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VARIABILITY OF NEAR-SURFACE ZOOPLANKTON OFF SOUTHERN CALIFORNIA, AS SHOWN BY TOWED-PUMP SAMPLING

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ABSTRACT

Variations in the density of near-surface populations of small copepods, large copepods, euphausiids, and chaetognaths are described for an area of 6,000 square miles off the coast of southern California from three cruises in the autumn of 1961 and two cruises in the autumn of 1962. Samples were collected with a towed pump at a depth of 5 m. Approximately 162 samples, each representing a 1-mile transect, were collected on each cruise.

Median densities for the cruises showed some significant differences for each species group. The frequency distribution of densities within the area on individual cruises varied from positive skewness at low general levels to relative symmetry at high general levels for the three crustacean groups, but was skewed at all levels for chaetognaths. Within sampling blocks of 20 square miles, the range of density varied with the median as $\log R = 0.35 + 0.8 \log M$. Range is greater than the median when the latter is less than 50, but less than the median when it is higher than 50.

Euphausiids and large copepods showed greater diurnal change than small copepods and chaetognaths.

Dry weight concentration of samples, averaged over all cruises, was 17.3 mg/m³ for the day period (0600-1800) and 25.1 mg/m³ for the night period. Most of the nighttime increase is attributable to the euphausiid group.

The three crustacean groups, and dry weight, showed significant inverse trends with temperature, but not with distance from land. The trends with temperature reflect events in 1961 but not in 1962.

These variations suggest that food potential of plankton for pelagic fishes may be appreciably greater than indicated by general averages for the area, depending on the degree of selectivity and orientation to small-scale features of distribution by the fishes.

Little is known about the effects of plankton variability on the distribution, movements, or rate of feeding of pelagic fishes which feed on plankton. It has been demonstrated experimentally (Ivlev, 1961) for some fishes that rate of feeding varies not only with average density but also with the degree of aggregation of food organisms in an area. Plankton density is known to vary diurnally (Cushing, 1951; King and Hida, 1954) as well as seasonally and annually, and there is evidence of aggregation in the variation for both small and broad spatial scales (Barnes and Marshall, 1951; Cassie, 1959, 1962, 1963). The plankton pump surveys reported here were undertaken to obtain information on variability and trends in variability for four plankton species groups commonly present in near-surface waters along the southern California coast. Though surveys were limited to

the autumn seasons of 2 consecutive years, the data should be a useful guide in evaluating the food potential of near-surface plankton distributions in the region.

COLLECTION OF SAMPLES

Samples were collected with the towed pump and shipboard filtering system described by O'Connell and Leong (1963). The 1.9-cm (3/4-inch) orifice of the pump pointed forward to achieve a coring orientation, and the rate of pumping (98 liters/min) exceeded the passive coring rate to produce in effect a 5.8-cm (2-inch) diameter coring cross-section. Operation of the system was essentially a matter of leaving the pump in tow and running throughout a cruise pattern with the incoming water stream diverted to the scuppers except while traversing sampling blocks, at which time the flow was directed through the filtering apparatus. The stainless steel filtering screen (105 μ mesh) retained

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virtually all organisms as small as 200μ in length (O'Connell and Leong, 1963) or 100μ in diameter (Leong, 1967). The upper size limit was less easily defined, but organisms as long as 14 mm were delivered at the filtering apparatus, though the large individuals were often mutilated.

Five cruises were carried out, three in September to November 1961 and two in the same period of 1962. For each cruise a pattern of 18 sampling blocks was selected from a possible 281 that covered an area of almost 6,000 square miles (Figure 1). To insure reasonably good coverage of the entire area, the population of

blocks was divided into three approximately equal subareas and a set of six blocks was selected at random from each. The blocks were occupied by the shortest practical track from northwest to southeast. Each cruise pattern required about 2.5 days of vessel time.

Each sampling block was 51.8 km^2 (20 square miles) in area, the only exceptions being some of the blocks adjacent to the coast or to islands. Nine 1.6-km (1-mile) samples were collected at each block in a continuous series along two connecting sides (Figure 1, insert) and were preserved in Formalin for laboratory processing.

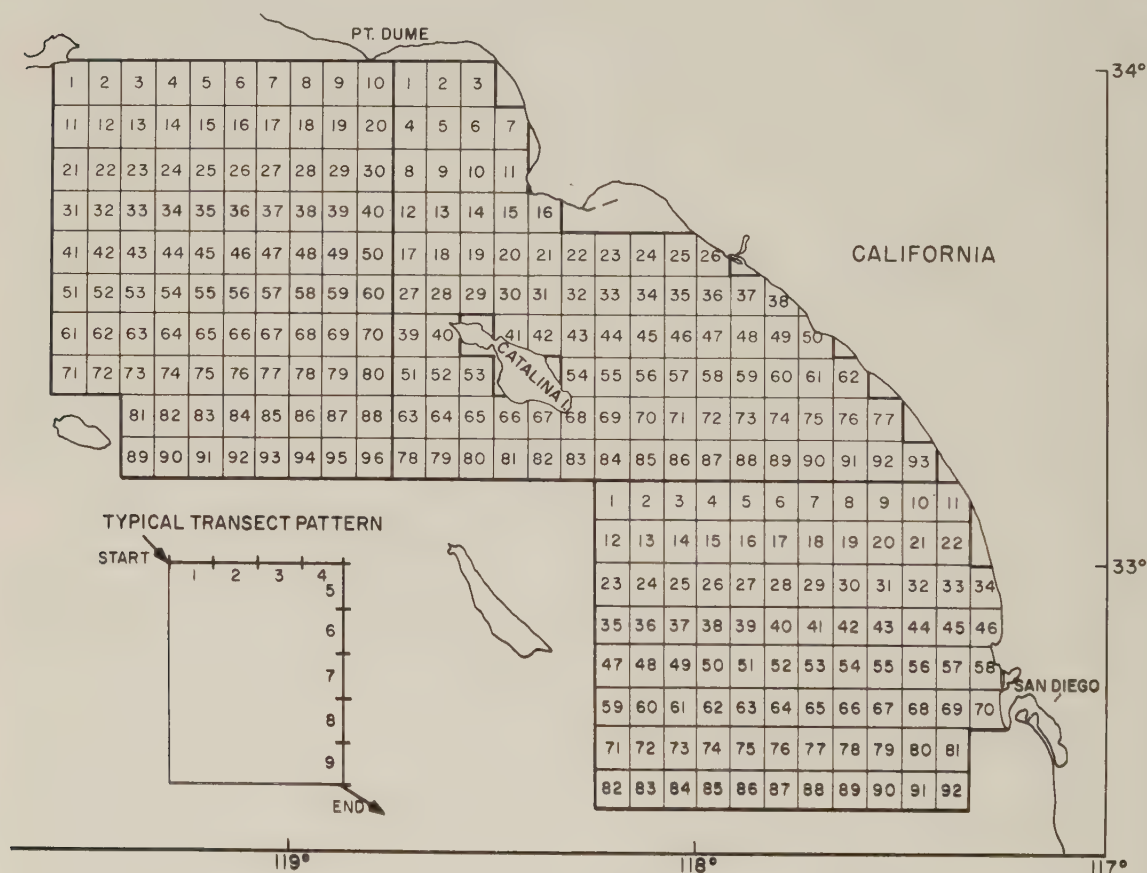


FIGURE 1.—The sampling area and entire population of sampling blocks. Six blocks were randomly selected from each of the three subareas for each cruise and occupied by the shortest route from north to south. Sampling blocks are 4×5 miles and the insert shows the manner of transect sampling. Four of the nine samples from each block were selected randomly for organism enumeration.

Consecutive 1.6-km samples were separated by shifting the incoming stream to a new filter every 6.5 min. Water volume, recorded for each sample from a meter in the incoming line, averaged 636.7 liters (standard deviation 47.8). Water temperature of the incoming stream was recorded at approximately the midpoint of each series of block samples. Continuous thermograph records indicated that surface water temperature did not change appreciably during the sampling of individual blocks.

SAMPLE PROCESSING

Four of the nine samples from each block were randomly selected for the estimation of numbers of organisms. Of the remaining five, one was chosen at random to be reserved for special purposes such as length and dry weight measurements of species groups, and the other four were pooled to obtain a dry weight value for the block. Estimates were standardized as quantities per m^3 on the basis of the actual volumes of water filtered in the samples.

Estimates for two size categories of copepods, 0.2 to 0.9 mm in length and all over 1.0 mm long, and for euphausiids and chaetognaths, were made by volumetric subsampling with replacement, i.e., each subsample was returned to the sample before the next subsample was drawn. The volumetric subsampling technique yields estimates of satisfactory precision only if organisms are randomly distributed in the sample container prior to the removal of each subsample. Simple stirring accomplished this for all species groups except the small copepods, probably because they were entangled in phytoplankton present in the samples. Random distribution was assumed to exist where the value s^2/x did not exceed $\chi^2/N-1$ for a series of subsamples (Holmes and Widrig, 1956). A random distribution was achieved for the small copepods by subjecting the sample to a few 1-sec bursts of rapid stirring in a Waring Blender.² However, because this treatment fragmented some larger organisms a two-step procedure was employed:

all organisms except the small copepods were estimated by subsampling following gentle stirring; the sample was then agitated in the Waring Blender, after which the small copepods were estimated by subsampling.

Estimates for the small copepods were always based on subsample counts totaling 200 to 300 from the sample. With the assumption of random distribution, the number in the sample should in all cases be within 15% of the estimate for $p = 0.05$ (Holmes and Widrig, 1956). More than half of the sample estimates for the other three species groups were based on counts of 30 or more, for which the number in the sample should have been within 40% of the estimate. For the remainder, where numbers counted were low, examination was not extended beyond subsamples totaling one-third the volume of the sample container, 2,000 ml.

In addition to the four species groups counted, the samples contained larvaceans and small invertebrate eggs (0.15-0.35 mm diameter), sometimes in moderately high numbers. Larvacean tails and heads were separated, however, and invertebrate eggs were not readily distinguishable from the latter. Cladocerans and polychaetes were generally absent or low in number, though each occurred in high numbers in a few samples. Fish eggs occurred rarely and in low numbers.

SIZE OF ORGANISMS

Length measurements for 10 day samples (0600-1800) and 10 night samples are summarized in Table 1 and Figure 2. Measurements were total length except for euphausiids, which were measured from the carapace behind the eye to the junction of the abdomen and telson. Data from day and night samples were pooled for small copepods and chaetognaths but not for large copepods and euphausiids, which showed appreciable size frequency differences for the two periods.

The length-frequency distribution for the small copepod group, composed largely of naupliar and copepodite stages, is nearly symmetrical, with the mean and the median close to the midpoint of the predetermined size range (0.20-0.99 mm long). Almost one-third of the organisms

² The use of trade names is merely to facilitate descriptions; no endorsement is implied.

TABLE 1.—Length data for species groups.

	Mean length	Median length	Minimum length	Maximum length
	mm	mm	mm	mm
Small copepods	0.58	0.56	0.20	0.99
Large copepods:				
Day	1.94	1.90	1.00	5.30
Night	1.75	1.61	1.00	4.20
Euphausiids:				
Day	2.3	2.1	0.6	9.7
Night	5.7	5.9	1.1	10.6
Chaetognaths	6.4	5.5	2.0	14.5

were between 0.5 and 0.6 mm in length. It is possible that the decline in numbers below the median length was partly the result of increasing escapement with diminishing size, but the escaping fraction, known to be negligible for sizes above 0.4 mm, is assumed to be relatively small for sizes down to 0.2 mm (O'Connell and Leong, 1963). On this assumption the length-frequency distribution is considered representative for this size range of copepods.

The large-copepod group shows a modal shift to smaller sizes at night, although the size range and degree of skewness are not markedly different for the two periods. Organisms less than 1.5 mm in length were largely copepodites, while

those between 1.5 and 3.0 mm were adult *Calanus helgolandicus* and *Paracalanus* sp., with *Centropages* sp. also present in the night samples. Those larger than 3.0 mm were largely *Rhincalanus* sp. in both day and night samples.

The euphausiid group, which appeared to be composed largely of *Euphausia pacifica*, though *Nyctiphanes simplex* and *Nematoscelis difficilis* were also in evidence, showed a marked shift to larger sizes at night, obviously the result of vertical migration. It is apparent from the sizes involved that the day samples were composed mostly of larval stages and the night samples of larval stages and juveniles, with few if any adults. The largest individuals in the samples were considerably smaller than the maximum total length for the species, 25 mm (Boden, Johnson, and Brinton, 1955).

The pump samples did show some evidence of fragmentation of larger euphausiids, and for this reason the size frequency distribution for the night period might be slightly biased in favor of the smaller sizes, and estimates of numbers sampled might be a little low.

Fragmentation probably involved far more juveniles than adults. Samples from opening-closing nets 1 m in mouth diameter taken in spring and summer off southern California (Brinton, 1962) showed adults to be scarce or absent in the upper 10 m during the night as well as the day. Juveniles were predominant at this stratum. Even at depths where adults were most abundant at night—40 m in one case and 140 to 280 m in another—they were only one-fifth and one-tenth as numerous as juveniles. Night-time oblique hauls with nets 1 m in mouth diameter off central Baja California indicated essentially the same kind of vertical distribution for euphausiid species in that area (Ahlstrom and Thrailkill, 1963). On the basis of such evidence it seems probable that the size-frequency distributions shown by the pump samples are reasonably representative of the day and night populations near the surface, though certainly not of the population in the entire water column.

The chaetognath group was composed largely, if not entirely, of *Sagitta euneritica*, and the size range is probably representative for the near-surface population sampled. The size range for

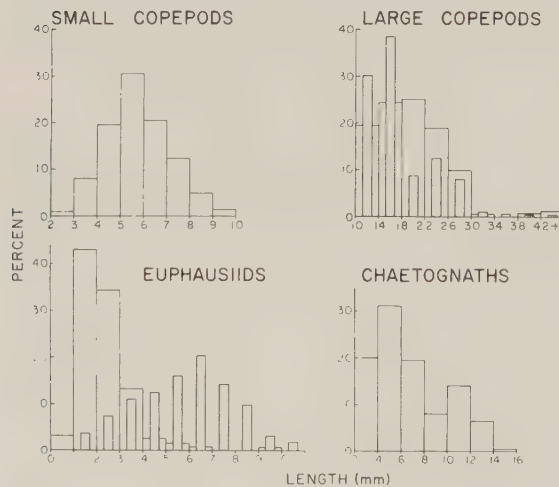


FIGURE 2.—Length-frequency histograms of organisms in four species groups, as determined from selected samples. For large copepods and euphausiids the wide bars show day frequencies and the narrow bars night frequencies.

the species is 1.0 to 15.5 mm in length (Alverino, 1961), and the samples contained individuals from 2.0 to 14.5 mm long.

VARIATION BETWEEN CRUISES

Table 2 shows characteristics of the frequency distributions of all 1.6-km samples by cruise and period of the day for the four species groups. Median densities are lower than mean densities in every instance but one, the nighttime period for small copepods on cruise 5, indicating that distributions for virtually all arrays show some degree of positive skewness. Differences between cruises are described in terms of the medians to avoid undue effects of extreme values that can arise in moment measures on nonnormal populations.

Nighttime median densities are higher than daytime medians on all cruises for small copepods and euphausiids, and on all but the fifth cruise for large copepods. However, variation was such that day and night medians differed significantly ($p < 0.05$, Tate and Clelland, 1957) in only one instance for small copepods, three instances for large copepods, and two instances for euphausiids. Though none of these groups can be considered to show consistently higher densities at night for the area as a whole, real differences occurred more often for the large copepods and euphausiids than for the small copepods. Day and night median densities for chaetognaths do not differ significantly for any of the cruises.

Daytime median densities do not differ significantly between cruises for chaetognaths or euphausiids, but those for small copepods and large copepods differ significantly ($p < 0.05$) for about half the comparisons. Nighttime medians (Figure 3) show a pattern similar to the daytime sets for the two copepod groups but show, in addition, a number of significant differences between cruises for the euphausiids and one difference for chaetognaths. Median densities of the small copepods were significantly higher for

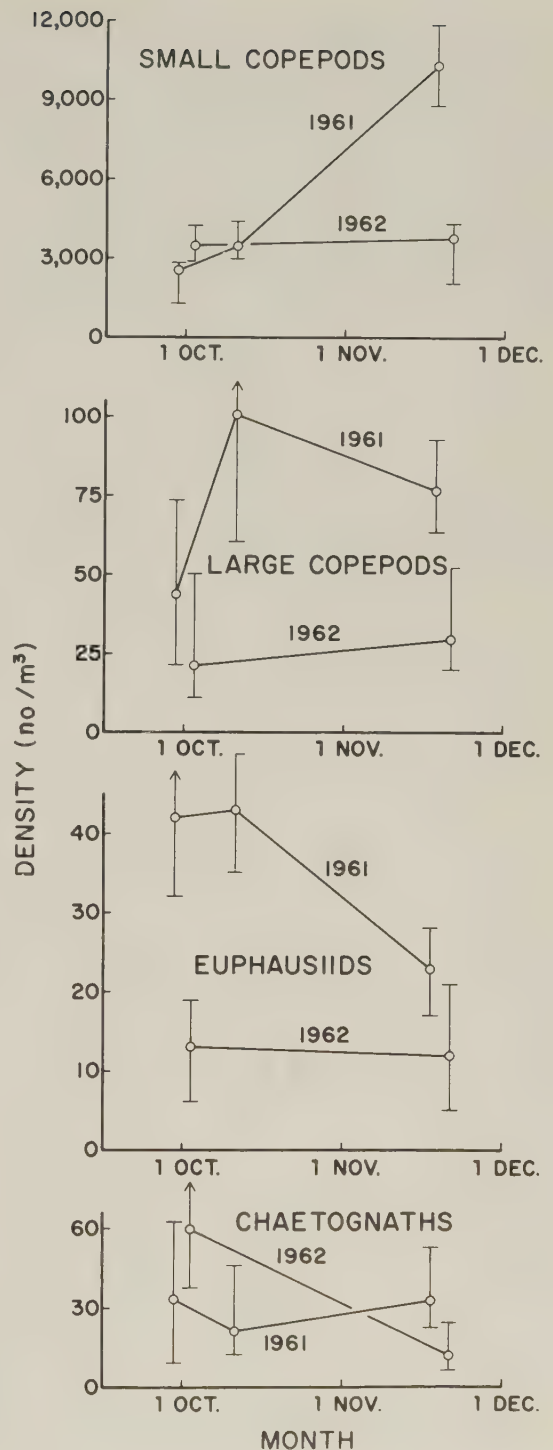


FIGURE 3.—The nighttime cruise medians for four species groups shown by cruise date. Vertical bars indicate the 95% confidence intervals.

TABLE 2.—Summary of density estimates (no/m³) of 1.6-km samples by cruise, constituent group, and period of day.

	Mean	s	Median	Confidence limits ¹ p = 0.95		Minimum	Maximum
				Lower	Upper		
Cruise 1, September 26-28, 1961, N = 32 day, 40 night							
Small copepods:							
Day	2,569.7	987.4	2,275.5	2,023	2,552	1,210	5,233
Night	2,983.9	1,356.2	2,542.0	2,252	2,792	1,075	7,103
Large copepods:							
Day	6.4	16.0	1.0	0	5	0	83
Night	72.8	95.5	42.5	22	73	0	500
Euphausiids:							
Day	6.7	9.4	3.0	2	8	0	38
Night	52.2	37.1	41.5	32	57	10	172
Chaetognaths:							
Day	84.2	122.9	21.0	8	105	2	488
Night	123.6	244.2	33.0	10	63	2	1,153
Cruise 2, October 9-11, 1961, N = 24 day, 40 night							
Small copepods:							
Day	3,379.0	1,773.1	3,034.5	2,217	3,527	1,550	7,532
Night	3,953.0	1,620.0	3,325.0	2,942	4,257	2,142	8,118
Large copepods:							
Day	47.3	104.5	1.0	0	17	0	440
Night	102.7	67.3	101.0	60	128	5	250
Euphausiids:							
Day	10.9	19.7	1.0	0	15	0	88
Night	48.2	31.7	42.5	35	50	7	145
Chaetognaths:							
Day	132.7	135.3	121.5	3	198	0	420
Night	51.8	61.9	21.0	13	47	2	280
Cruise 3, November 16-18, 1961, N = 28 day, 40 night							
Small copepods:							
Day	8,679.0	3,969.7	7,381.5	6,107	10,208	3,752	19,868
Night	10,577.8	3,060.7	10,281.0	8,673	11,812	4,948	17,568
Large copepods:							
Day	18.3	21.1	10.0	5	18	0	92
Night	83.0	42.9	77.0	63	95	10	183
Euphausiids:							
Day	15.5	13.9	13.0	5	22	0	48
Night	39.5	50.8	23.0	17	28	2	243
Chaetognaths:							
Day	73.5	104.5	36.0	32	42	12	470
Night	44.5	38.8	32.5	23	50	3	188
Cruise 4, October 1-3, 1962, N = 43 day, 26 night							
Small copepods							
Day	2,718.1	1,748.8	1,993.0	1,739	2,771	456	8,190
Night	3,524.1	1,087.1	3,504.0	2,854	4,179	1,477	5,560
Large copepods:							
Day	21.3	19.6	18.0	10	22	0	90
Night	33.6	29.5	21.5	11	50	3	99
Euphausiids:							
Day	8.2	9.1	4.0	3	10	0	39
Night	16.0	14.3	13.0	6	19	1	58
Chaetognaths:							
Day	116.7	164.6	57.0	35	133	9	993
Night	86.9	71.6	60.0	38	113	13	257
Cruise 5, November 19-21, 1962, N = 32 day, 31 night							
Small copepods:							
Day	3,728.2	2,277.8	3,487.0	2,235	4,328	1,654	11,613
Night	3,505.3	1,962.1	3,750.0	2,054	4,223	796	9,476
Large copepods:							
Day	121.5	252.0	49.5	29	70	0	1,220
Night	41.1	35.6	30.0	19	52	1	154
Euphausiids:							
Day	9.8	11.0	6.5	1	13	0	48
Night	24.4	34.4	12.0	5	21	0	163
Chaetognaths:							
Day	32.2	20.8	28.5	19	41	3	94
Night	21.0	20.2	12.0	7	25	0	72

¹ Confidence limits are for medians (Tate and Clelland, 1957).

each successive cruise in 1961, and the median for the last cruise in this year was more than three times as high as any of the others. Median densities of the large copepods were two to four times as high for the last two cruises in 1961 as for the other cruises. Euphausiid medians were about twice as high for the first two cruises of 1961 than for the other cruises. The chaetognath median for the last cruise of 1962 was significantly lower than for the first cruise of that year, but no other differences can be distinguished.

In summary, the three crustacean groups showed real differences in density near the surface at night for the area as a whole. All showed differences of three to four times between the 2 successive years. The euphausiids and small copepods also showed real differences of about two and three times within the first of the 2 years.

VARIATION WITHIN THE AREA

The 1.6-km samples were taken in block clusters so that small-scale variability could be described in respect to variability over the entire area. The possibilities of comparison are limited, however, because parametric analyses were avoided. The necessary assumptions about frequency distribution of sample estimates could not be satisfied for the present data. Frequency distribution of block medians (or means) is variable between cruises.

FREQUENCY DISTRIBUTION OF BLOCK MEDIAN

Table 3 shows the frequency distribution of block medians for the day and night periods of each cruise. The distributions for each species group vary noticeably, but there are similarities

TABLE 3.—Frequency distribution of block medians by cruise and day (D) or night (N) period.

Class (no./m ³)	1D	1N	2D	2N	3D	3N	4D	4N	5D	5N
Small copepods:										
1-3,000	6	7	3	4	--	--	8	3	4	4
3,001-6,000	2	3	2	5	--	--	3	4	4	5
6,001-9,000	--	--	1	1	5	4	--	--	--	--
9,001-12,000	--	--	--	--	1	2	--	--	1	--
12,000-15,000	--	--	--	--	1	4	--	--	--	--
Category	1	1	2	2	3	4	1	2	2	2
Large copepods:										
0	3	--	3	--	--	--	--	--	--	--
1-50	5	5	2	3	7	1	11	5	6	7
51-100	--	3	--	2	--	7	--	2	1	1
101-150	--	1	--	3	--	2	--	--	--	1
151-200	--	--	--	2	--	--	--	--	1	--
201+	--	1	1	--	--	--	--	--	1	--
Category	1	3	1	4	1	4	1	2	3	2
Euphausiids:										
0	2	--	2	--	1	--	2	--	1	--
1-25	5	2	3	2	5	5	9	6	8	7
26-50	1	3	1	4	1	3	--	1	--	1
51-75	--	3	--	3	--	1	--	--	--	--
76-100	--	2	--	1	--	--	--	--	--	1
101-125	--	--	--	--	--	--	--	--	--	--
126-150	--	--	--	--	--	--	--	--	--	--
151+	--	--	--	--	--	1	--	--	--	--
Category	1	3	1	3	1	2	1	2	1	2
Chaetognaths:										
1-50	5	6	2	7	5	7	4	2	8	9
51-100	1	2	1	1	1	2	3	2	1	--
101-150	--	--	--	1	1	1	3	2	--	--
151-200	--	--	2	1	--	--	--	--	--	--
201-250	1	1	--	--	--	--	--	1	--	--
251-300	1	--	1	--	--	--	--	--	--	--
300+	--	1	--	--	--	--	1	--	--	--
Category	1	1	3	2	2	2	3	3	2	2

among them suggestive of trends with the general level of density. To define the trends, the distributions for each species group were pooled into three or four categories on the basis of the extent of concentration in specific frequency classes and the extent of dispersion over all classes. Category designations are indicated in the table, and percentage frequency histograms are shown for each category in Figure 4. Number of blocks, number of night and day periods, and the range of cruise medians (from Table 2) are given for each histogram.

The histograms suggest the same kind of trend for the three crustacean groups: a shift from distribution almost entirely restricted to the lowest classes when general area level is low, through distribution of greater positive skewness for intermediate levels of area density, to symmetrical distribution as blocks of low median value disappear at the higher levels of area density. The chaetognath distributions were more difficult to classify, but it appears that frequency distribution is appreciably skewed at all levels of area density for this group.

The differences in the trends for the four species groups are also illustrated by the extent of the overlap between the distributions for the highest and lowest categories in the figures. There is no overlap for small copepods, perhaps 50 to 75% overlap for large copepods and euphausiids, and complete overlap for chaetognaths. These differences suggest that when the overall area median is at one extreme, the possibility of blocks with medians at the other extreme is greatest for the chaetognaths, least for the small copepods, and intermediate for the large copepods and euphausiids.

The existence of such trends in frequency distribution indicates that, at least for the crustaceans, no one statistical distribution would satisfactorily fit all the data sets; nor would any single normalizing transformation be uniformly effective for the different data sets. Without normalized distributions, even the interpretation of coefficients of variation would be difficult in comparing cruise periods. It may be noted, however, that when all frequency distribution cate-

gories are pooled, the distributions for the four species groups show similar degrees of skewness. The total block array for each species group is approximately normalized by log transformation.

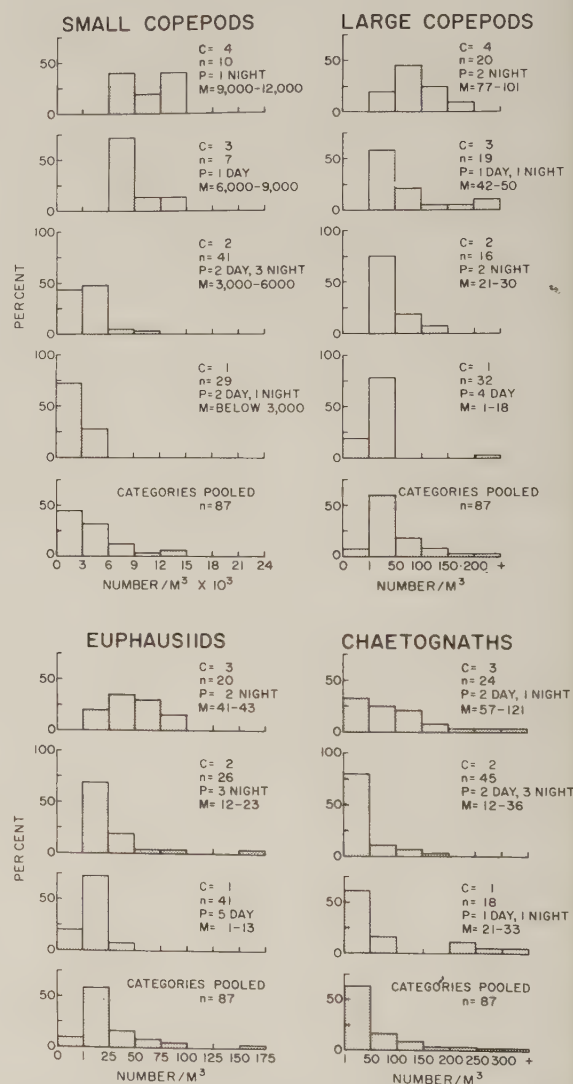


FIGURE 4.—Percent frequency distribution histograms for four species groups as pooled on the basis of similarity. Range of sample medians (M) (from Table 2), number of day and night periods (P), and number of sampling blocks (n) represented by each histogram category (C) is shown. The histogram at the bottom of each set shows the distribution of all block medians from all cruises.

RELATION OF RANGE TO MEDIAN IN SAMPLING BLOCKS

Within block variation is indicated for each species group and for all species groups together by regression $\log R = a + b \log M$ (Table 4), where R = block range and M = block median. The slopes, b , for the five equations do not differ significantly from each other ($p = 0.05$). All are significantly greater than 0 ($p = 0.01$), and all but that for small copepods are significantly less than 1.0 ($p = 0.05$). The intercepts are all significantly greater than 0, again except for the small copepods, but they do not differ significantly from each other ($p = 0.05$). In view

TABLE 4.—Estimated coefficients for regressions of log block range on log block median for each species group and all species groups combined.¹

Species group	<i>N</i>	<i>a</i>	<i>b</i>	<i>s</i>	<i>r</i>
Small copepods	87	-.18	.95**	.40	.53**
Large copepods	81	.51	.72**	.36	.74**
Euphausiids	79	.38	.72**	.30	.79**
Chaetognaths	86	.26	.87**	.33	.82**
Combined	333	.35	.80**	.35	.93**

¹ *N* = number of sampling blocks; *a* = intercept; *b* = slope; *s* = standard deviation about the line; *r* = correlation coefficient.
** $p = 0.01$.

of the similarities, the regression for all groups combined (Figure 5) is a satisfactory description of the average relation of block range to block median for each of the species groups.

The regression for all groups combined indicates that average block range increases with block median but not proportionately. The expected ranges for different medians are:

Median	1	10	50	100	1,000	10,000
Range	2	14	51	89	562	3,548

Thus range will tend to be greater than the median when the latter is below 50, but appreciably less than the median when the median is above 100. Small copepods are the only group with consistently high medians, and they also have the greatest standard deviation about the line. This suggests that, while the ratio of range to median is lower, on the average, for this group, it also tends to be more variable than for the other groups.

The relation of block range to the total variation within the area is suggested by the magnitude of block ranges relative to the class in-

tervals of the frequency distributions shown earlier (Table 5). In the case of small copepods, block ranges only slightly exceed the size of class intervals for the two highest classes in the distribution. For the other three species groups, block ranges exceed the class intervals for all but the lowest class. Ranges tend to be spread over three or more class intervals for the upper halves of the euphausiid and chaetognath distributions. Even though the highest classes tend to be rare, it can be seen that ranges extending over two or more class intervals would not be uncommon for large copepods, euphausiids, and chaetognaths.

TABLE 5.—The number of class intervals encompassed by the range for the midpoint of each class interval in the frequency distribution for each species group. The 0-1 class is excluded and other classes are numbered consecutively.

Frequency class	Small copepods	Large copepods	Euphausiids	Chaetognaths
1	0.1	0.6	0.7	0.6
2	0.3	1.4	1.6	1.4
3	0.9	2.1	2.4	2.1
4	1.2	2.8	3.2	2.8
5	1.5	3.4	3.9	3.4
6	--	--	4.6	4.0
7	--	--	5.2	4.6

DIURNAL VARIATION

It is evident from the differences in day and night sets of data that density level near the surface is influenced by diurnal vertical movements, particularly for the larger crustacean groups. The pattern of change is indicated for each of the species groups by medians for 4-hr time intervals (Figure 6). A sequence of change is most apparent for the large copepods and euphausiids, the lowest medians occurring between 1000 and 1400 hr and the highest between 1800 and 0200 hr. The increase in the evening appears to be more rapid than the decrease in the morning for both groups.

The small copepods show a pattern similar to that for large copepods, but much weaker. The highest time interval median is almost 10 times the lowest for large copepods but less than 2 times the lowest for small copepods.

Chaetognaths show only slight evidence of diurnal change. The peak between 0600 and

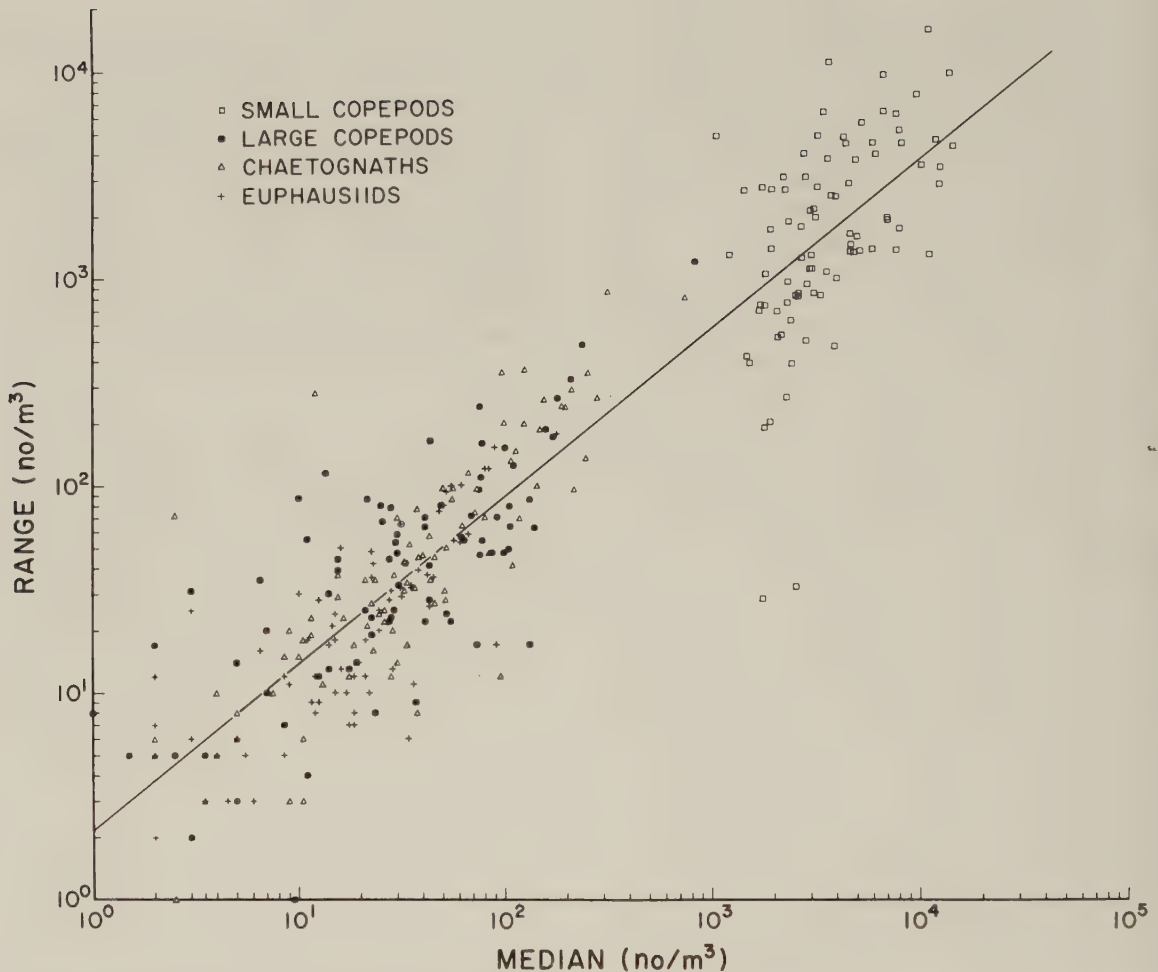


FIGURE 5.—The relation of sampling block range to sampling block median for the four species groups combined.

1000, which is obviously responsible for the generally higher day-period densities shown elsewhere, may indicate an upward and a downward movement in the morning.

The spread of block medians tended to be associated with the time-interval medians for euphausiids but not for the other species groups. The euphausiids showed both high and low block medians at night but only low medians during the middle of the day. Large copepods showed a similar distribution except that three of the highest four block medians in the series occurred between 1000 and 1800 hr. Small copepods and

chaetognaths showed high and low medians in all time periods.

CORRELATIONS BETWEEN DENSITIES OF THE FOUR SPECIES GROUPS

The data were examined for association between the densities of species groups over the area by calculating rank-difference correlation coefficients (Tate and Clelland, 1957) for the nighttime block medians of each cruise (Table 6). Daytime blocks were excluded to reduce the component of correlation that would result from

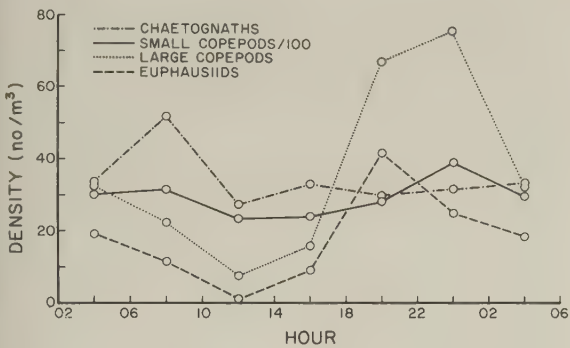


FIGURE 6.—The relation of median density to time of day for four species groups. The points are medians for block densities in 4-hr intervals. The small copepod medians were divided by 100 to put them on the same scale as the others.

TABLE 6.—Rank difference correlation coefficients for median block densities of four species groups for the night periods of five cruises.

	Cruise				
	1	2	3	4	5
Small copepods : large copepods	-.45	.31	.25	.71	.20
Small copepods : euphausiids	-.09	-.20	.59*	.14	.87**
Small copepods : chaetognaths	.26	-.36	-.43	-.42	.30
Large copepods : euphausiids	.10	.04	.71*	.14	.29
Large copepods : chaetognaths	.02	.29	.20	.21	.73*
Euphausiids : chaetognaths	.42	.12	-.23	.00	.53
Number of night blocks	10	10	10	7	9

* $p = 0.05$.
** $p = 0.01$.

the parallel patterns of diurnal change in the crustacean groups.

The coefficients for each of the six species group combinations varied widely among the five cruises, with only four of the entire 30 coefficients indicating significant correlations. It appears that, while occasional correlations can be expected to occur over the area, consistent trends of association in density do not occur among these four species groups near the surface at night.

DRY WEIGHT VARIATION

Dry weight concentrations are summarized by cruise period in Table 7 and by weight class for all cruises in Figure 7. Low and high values occur both night and day, but there is clearly a shift to higher values at night.

The sample concentrations may underestimate the true concentrations by as much as 15 or 20%. Ahlstrom and Thrailkill (1963) showed that for copepods dry weight decreased about 15% after Formalin preservation. Lasker (1966) showed that dry weight of euphausiids was about 35%

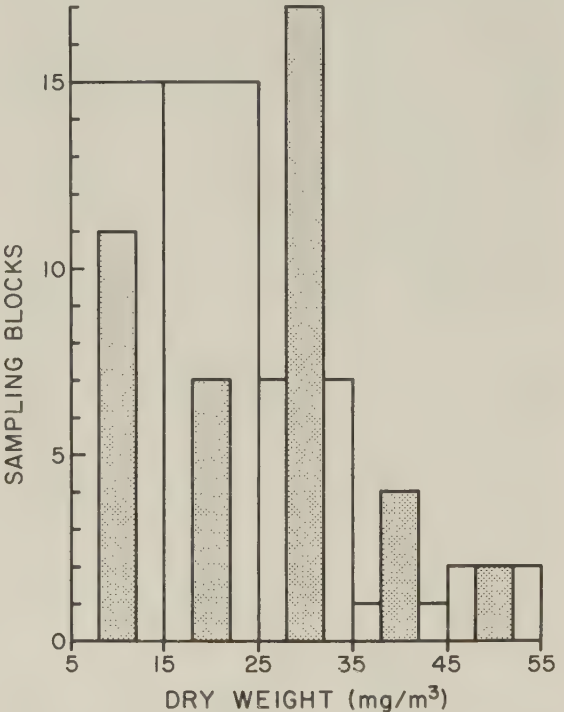


FIGURE 7.—Dry weight frequency distribution of all sampling blocks. The wide bars show day frequencies and the narrow bars night frequencies.

TABLE 7.—Summary of sample dry weight concentration (mg/m³) by cruise and day (D) or night (N) period.

	1D	1N	2D	2N	3D	3N	4D	4N	5D	5N
Mean	13.04	20.87	18.02	22.24	22.94	34.01	22.67	24.51	18.44	25.58
Median	15.00	18.32	14.51	21.03	22.76	31.80	15.24	24.72	15.27	23.23
Minimum	5.81	9.36	5.54	13.54	14.17	25.79	7.02	9.68	8.46	9.54
Maximum	20.85	34.00	43.74	30.57	30.42	49.59	56.90	34.62	32.11	52.42
Number blocks	7	9	6	7	7	9	11	7	9	9

lower for preserved than for fresh material. These groups were prominent in the pump samples, and dry weight determinations were made after long Formalin preservation. The pump samples undoubtedly contained a higher proportion of small copepod forms than the net samples of Ahlstrom and Thrailkill (1963), and it is possible that dry weight loss in Formalin is less for the smaller than for the larger individuals of this group.

As a basis for estimating the contributions of different species groups to dry weight concentration, dry weight factors were determined for large copepods, euphausiids, and chaetognaths by sorting known numbers of each from a few representative samples for drying and weighing. The resulting values are given in Table 8. The factor given for small copepods, which would have been difficult to separate in sufficient numbers and purity for a direct determination, was inferred from data given by Marshall and Orr (1955) for *Calanus finmarchicus* and *C. helgolandicus* in eastern Atlantic waters. They showed that *Calanus* stage V, at an average length of 2.5 mm, have a dry weight of about 300 mg/1000 organisms, and that according to Bogorov (1933) one stage V organism is equal in dry weight to two stage IV, 11 stage III, 42 stage II, and 60 stage I organisms. The average lengths of these stages are given as 2.1 mm, 1.65 mm, and 0.94 mm, and the average length of nauplii is given as 0.585 mm, which is the same as that of small copepods in the present study. Large copepods from the present study show an average length intermediate between those given for stages IV and III above,

TABLE 8.—Dry weight (DW) and ash weight (AW) determinations (mg/1000 organisms) for species groups in selected samples.

	DW	AW	AW/DW
	mg/1000	mg/1000	%
Small copepods	12.5	--	--
Large copepods:			
Day	54.64	3.48	6.36
Night	49.88	2.94	5.89
Euphausiids:			
Day	42.03	3.29	7.82
Night	293.26	14.51	4.95
Chaetognaths	23.5	1.30	5.51

¹ Not determined by direct measurement. See text.

suggesting a ratio of six large copepods to one stage V, or a dry weight of 50 mg/1000 organisms, which is very close to the actual determinations. No dry weight equivalent is given by Bogorov for nauplii, but extrapolation of his series against average lengths suggests that 120 nauplii per stage V *Calanus*, or 2.5 mg/1000 nauplii, would be a conservative estimate.

Dry weight concentrations were calculated for each species group in each block from the dry weight factors and from the medians of numerical estimates for the blocks. The values for species groups were summed to produce a "calculated" dry weight concentration for each block. These are compared to the measured dry weight concentrations in Figure 8. All the data together tend to cluster around the line of equal value (slope 1.0), and each of the different

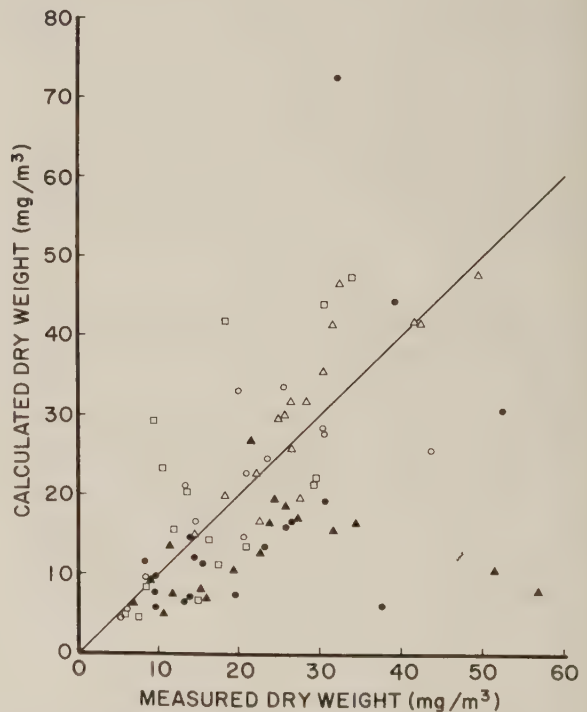


FIGURE 8.—The relation of calculated to measured dry weight concentration for all sampling blocks. Calculated dry weights were derived from species group density estimates and dry weight factors (Table 6). The line indicates equality of the two scales □ Cruise 1; ○ Cruise 2; △ Cruise 3; ▲ Cruise 4; ● Cruise 5.

cruise sets has slopes similar to the line. Variation is wide, but there are only four serious discrepancies. Whatever the reasons for these discrepancies, the four blocks were excluded from calculation of the relative contributions of species groups to dry weight concentrations for day and night cruise periods.

Table 9 shows the averages of dry weights calculated for each species group for each cruise period, and for all cruise periods pooled by day and night. "Calculated" sample concentrations, obtained by summing the values for the four species groups, are compared with the average measured concentrations for the cruise periods in the last two columns. Though the calculated concentrations are lower than the measured concentrations for all day periods, and higher than the measured concentrations for three of the night periods, the two sets show reasonably good agreement, as do the day and night averages for all cruises together.

The measured sample concentrations for all cruises pooled suggest that, on the average, dry weight was 31% higher at night than during the day. The calculated concentrations suggest that it was 48% higher. Calculated values for the species groups show that the dry weight increase at night is largely attributable to increases in the euphausiid group, with lesser increases in the large copepods and also the small copepods. Euphausiids were responsible for more than one-third of dry weight concentration

at night, on the average, and small copepods for less than half of it. During the day, on the other hand, small copepods accounted for about three-fourths of dry weight concentration, with most of the remainder divided between large copepods and chaetognaths.

VARIATION WITH TEMPERATURE AND DISTANCE FROM LAND

The data for each of the four species groups, and for dry weight, were examined for possible relationships with the independent variables, temperature, miles from nearest land (including islands), and miles from nearest point on the mainland. Regressions were in the form $\log Y = a + bX$, where X = the independent variable and Y = the dependent variable. Small copepods, large copepods, and dry weight showed significant trends with temperature, and chaetognaths showed significant trends with distance from land (Table 10). For euphausiids, night values alone, as well as day and night values together, were tested but neither demonstrated significant trends.

The two copepod groups and dry weight all show an inverse relation with temperature, but in all cases the trends are largely attributable to changes occurring in 1961, as a comparison of nighttime cruise medians with average cruise temperature shows (Table 11). The decline in

TABLE 9.—Calculated average dry weight fractions (mg/m^3) and percentages for species groups by cruise and day (D) or night (N) period.

	Small copepods		Large copepods		Euphausiids		Chaetognaths		Average sample dry weight	
	mg/m^3	%	mg/m^3	%	mg/m^3	%	mg/m^3	%	Calculated mg/m^3	Measured mg/m^3
1D	6.46	70.9	0.26	2.9	0.26	2.9	2.13	23.4	9.11	13.04
1N	7.70	26.1	3.49	11.8	14.88	50.5	3.41	11.6	29.48	20.87
2D	8.54	61.4	2.18	15.7	0.36	2.6	2.84	20.4	13.92	18.02
2N	9.29	35.7	4.08	15.7	11.83	45.5	0.79	3.0	25.99	22.24
3D	20.07	88.3	0.73	3.2	0.63	2.8	1.29	5.7	22.72	22.94
3N	25.74	68.7	3.70	9.9	6.99	18.7	1.03	2.7	37.46	34.01
4D	6.05	62.6	0.97	10.0	0.35	3.6	2.30	23.8	9.67	15.66
4N	8.88	51.0	1.50	8.6	4.95	28.4	2.07	11.9	17.40	24.51
5D	7.56	66.4	2.93	25.7	0.28	2.5	0.61	5.4	11.38	16.72
5N	8.52	47.7	2.09	11.7	6.86	38.4	0.41	2.3	17.88	24.09
Average D	9.74	72.9	1.41	10.6	0.38	2.8	1.83	13.7	13.36	17.28
Average N	12.03	46.9	2.97	11.6	9.10	35.5	1.54	6.0	25.64	25.14

TABLE 10.—Regressions of median block densities and dry weight concentrations on temperature and distance from land.¹

Regression	N	a	b	s	r
Small copepods on temperature	83	5.38	-.105*	.23	.54**
Large copepods on temperature	78	3.23	-.101*	.52	.26*
Dry weight on temperature	78	2.46	-.067**	.22	.40**
Chaetognaths on distance to mainland	85	1.99	-.013**	.53	.31**
Chaetognaths on distance to nearest land	85	2.02	-.020*	.54	.22*

¹ N = number of sampling blocks; a = intercept; b = slope; s = standard deviation about the line; r = correlation coefficient.

* p = 0.05.

** p = 0.01.

TABLE 11.—Average temperatures, median copepod densities, and median dry weight concentrations for the night periods of each cruise.

Cruise date	Temperature	Small copepods	Large copepods	Dry weight
	° C	no./m ³	no./m ³	mg/m ³
9/27/61	18.9	2,542	42	18
10/10/61	18.2	3,325	101	21
11/17/61	15.9	10,281	77	32
10/ 2/62	18.6	3,504	21	25
11/20/62	16.1	3,750	30	23

water temperature was approximately the same in both years. The copepod values and dry weight consequently show a strong inverse relation with temperature and date for 1961 but not for 1962.

The chaetognaths show inverse trends with distance from the mainland and from nearest land, but the former is the more significant of the two. The relationship with distance to nearest land includes many of the distance measurements to the mainland, of course, and it is possible that these are largely responsible for the significant relationship with distance to nearest land. The geographical distribution of all chaetognath block medians (Figure 9) shows that distance from the mainland is the more pertinent independent variable. Low densities occurred at all distances beyond 7 miles, whereas the highest densities did not occur farther offshore than 14 miles, with a single exception. It can be seen that density was far more variable near the mainland than offshore.

DISCUSSION

The food potential of plankton for pelagic fishes depends on the relation between the average density of some or all species groups over an area and the rate at which the fishes can feed on these species groups. Since median density of the more common species groups varies widely within the space of a few months for the area surveyed in this study, it is probable that food potential of near-surface plankton off southern California fluctuates appreciably within short time intervals. However, there were marked small-scale variations in the distributions of densities associated with the general area levels, and the general level would not be an appropriate index of food potential if fishes tend to orient to small-scale features of distribution.

Although the association of range with median for sampling blocks of 51.8 km² demonstrates that densities vary, sometimes widely, within the blocks, the medians of blocks of this or some similar size probably constitute a scale of sufficient resolution for assessing the food potential of plankton over a large area. Maximum values within the blocks are not likely to be impressively greater than the median. If the median of large copepods is 175/m³, the block can be expected to have, on the average, a maximum density of 245/m³ representing an area of 2.6 km² (1 square mile) within the block. If the median of euphausiids is 90/m³, the block can be expected to have a maximum density of 130/m³ for 2.6 km². Blocks with such medians are usually rare, and the medians, as well as the maximum values, are likely to be considerably higher than the densities in most blocks in the area. The medians would slightly underestimate the food potential of such blocks only if plankton feeding fishes tend to orient to the highest densities within the space of 20 square miles.

The distributions of sampling block medians, which were skewed unless general area level was very high, suggest that even under the poorest general conditions relatively high densities of organisms are likely to exist in some small portion of the survey area. Small copepods, for example, showed a few occurrences of blocks with medians above 6000/m³, and one

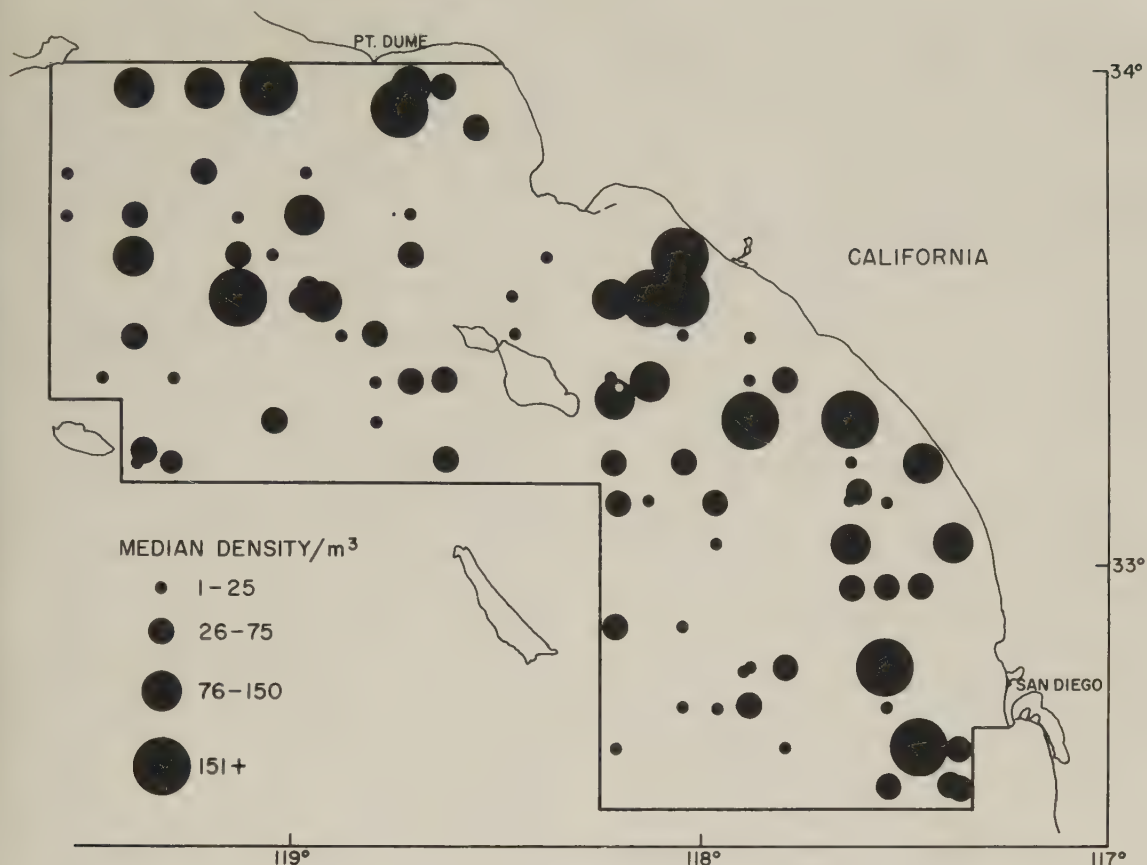


FIGURE 9.—Geographical distribution of chaetognath medians for all blocks on all cruises.

with a median above $9000/\text{m}^3$, when the general area median was about $3000/\text{m}^3$. Large copepods showed a few occurrences of blocks with medians well above 200 when the general area median was $50/\text{m}^3$ or less. Euphausiids showed occurrences of blocks with medians above $75/\text{m}^3$ when the general area median was between 25 and $40/\text{m}^3$. It seems probable, in other words, that high crustacean densities are always present somewhere in the area. At the lower general levels they would be scarce but perhaps as much as three times higher than densities over most of the area.

The data indicate that higher densities of chaetognaths are most likely to occur near the mainland, but they failed to demonstrate such trends for the three crustacean groups. They

indicate only that the crustaceans, and dry weight, may sometimes be at higher levels where and when temperatures are relatively low. Regressions with temperature show, for example, that the density of small copepods was $6400/\text{m}^3$ in 15°C water, on the average, as compared with $2400/\text{m}^3$ in 19°C water, and that dry weight concentration was $29\text{ mg}/\text{m}^3$ in 15°C water, but only $15\text{ mg}/\text{m}^3$ in 19°C water. Since variation associated with these trends is wide, it can only be concluded that water of low temperature may sometimes, though not always, contain much higher standing crops of zooplankton than are likely to be found in warmer water in the survey area.

Dry weight factors were determined for the different species groups because it is impossible

to estimate the relative nutritional value of such groups in the plankton on the basis of organism counts alone. In general, the near-surface zooplankton had a dry weight equivalent of about 25 mg/m³ at night, which was approximately 30% greater than the average daytime level. Small copepods were the dominant fraction during the day and increased only slightly at night. Most of the nighttime increase in dry weight is attributable to the appearance of euphausiids, which were estimated to have a dry weight equivalent of 9 mg/m³, on the average, as compared with less than 1 mg/m³ during the day. There was some deviation from this general pattern among the five cruises. Differences between and within sampling blocks were not described in terms of dry weight. The general differences are enough to show that the nutritional potential of the plankton, in terms of dry weight for any set of samples, would depend on the extent to which fishes do or do not feed selectively.

However they are interpreted, it must be noted that the dry weight equivalents of the samples taken in this survey, aside from the possible loss of weight in Formalin preservation, may not represent the whole of the biomass utilized by some plankton feeding fishes near the surface. They contained almost no zooplankters smaller than 0.2 mm in length and relatively little phytoplankton. The comparison in Table 12 indicates that such smaller organisms may constitute a considerable fraction of the biomass.

Beers and Stewart (1967) sampled the euphotic zone with a towed pump on a line of stations off San Diego. Water was strained successively through 202-, 103-, and 35- μ cloths to estimate quantities below each cloth. Leong and O'Connell (1969) estimated from the resulting data

that phytoplankton and zooplankton passing through the 103- μ cloth represented average dry weight concentrations of 25 and 3 mg/m³. The 103- μ cloth was approximately the same mesh size as the filtering screens used in the present survey. The material retained between the 103- and 202- μ cloths of Beers and Stewart is here judged to approximate the size range, 0.2 to 1.0 mm, and the numerical estimate for their innermost station, when converted to dry weight by the constant, 2.5 mg/1000 organisms, yields a concentration similar to the daytime average for crustaceans in this size range in the present surveys.

It appears that material smaller than that collected in the present survey might represent a dry weight approximately two to three times as great as that that was collected. Adjustment for the smaller organisms in questions of nutritional potential would depend on selectivity in the feeding of various fishes.

It is difficult to judge whether the dry weight values attributed to organisms larger than 1 mm in the above comparison fully represent the biomass of larger plankton organisms near the surface that can be utilized by plankton feeding fishes. Such fishes probably take crustaceans larger than collected by the pump when opportunity arises. It can only be restated that euphausiids larger than those sampled are relatively rare close to the surface. Five of the 1-m-mouth-opening-net tows taken by Ahlstrom and Thrailkill (1963) through the upper 100 m or so were composed largely of crustacean material. From the data given they were estimated to represent dry weight concentrations averaging 3.5 mg/m³. The figure is in the range of day and night concentrations for the pump samples, but the comparison is uncertain because of differences in the location as well as the depth of sampling.

The above discussion implies that estimating the food potential of plankton for fishes must depend as much on information concerning the feeding behavior of the fishes as on information concerning the abundance and variability of the plankton. The data given here on plankton variability are intended as a basis for interpreting hypotheses that may arise from laboratory or field studies of feeding behavior.

TABLE 12.—Comparison of dry weight values (mg/m³) for different length ranges of planktonic organisms in two towed pump studies.

	Period	Group	Length range (mm)		
			<0.2	0.2-1.0	>1.0
Beers and Stewart (1967)	Day only	Phytoplankton	25.1	0	0
		Zooplankton	2.9	8.9	0
Present survey	Day	Crustacean	0	9.7	1.8
	Night	Crustacean	0	12.0	12.1

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NOTE

PREDATION ON JUVENILE PACIFIC SALMON BY A MARINE ISOPOD

Rocinela bellicepts pugettensis
(CRUSTACEA, ISOPODA)

Observations were made of predation by a marine isopod on both captive and wild Pacific salmon. Pacific salmon are known to be hosts to a number of ectoparasites, especially the Copepoda. However, we have been unable to find any reported incidents of predation or parasitism of Pacific salmon by the Isopoda.

In July 1969, we began a series of experiments in Puget Sound to evaluate the feasibility of saltwater rearing of Pacific salmon within floating pens. Stocks of young salmon were weighed and measured several times during the year at the Manchester, Wash., experimental station of the National Marine Fisheries Service and routinely examined for ectoparasites visible to the naked eye. A large Branchiuran parasite, *Argulus* sp., was found on a 600-g chinook salmon (*Oncorhynchus tshawytscha*); the Branchiuran was removed from the fish and kept for study. Later, another Branchiuran was found in the same body position on this fish. There were no other visible ectoparasites.

In March 1970, we began collecting zooplankton to provide live food for both salmon fry and newly metamorphosed flatfish. Collections were made during the night; a surface light was used to attract the plankton and an airlift pump to draw them into a net suspended at the sea surface.

The collected organisms were poured into a tank containing over 1000 young pink salmon (*O. gorbuscha*), 30 to 45 mm long. Among the unsorted plankton were 8 or 10 isopods of the same species. The pink salmon fed vigorously on large numbers of amphipods in the tank, whereas the isopods alternately rested on the sides of the tank and swam about in random patterns. One isopod quickly attached itself to a young pink

salmon. Within minutes, two more of the young salmon were similarly attacked. The fish became distressed, swam erratically about the tank, and drifted listlessly to the bottom, where they died within a few minutes after settling. The point of attack by the isopod on each young salmon was lateral, just above or slightly posterior to the pelvic fin. Small, but deep wounds that penetrated the body wall were found on each affected fish. The gut of each of the isopods removed from the dead fish contained blood.

One of the isopods was placed in another tank containing several dozen coho salmon (*O. kisutch*), slightly larger than the pinks (100-140 mm long). An hour later, the isopod was found firmly attached to the head of one fish, immediately posterior to and between the eyes.

Isopods are now removed before any plankton are fed to our fish. If we should overlook even the smallest of these isopods, we can expect either death or injury to some fish.

We examined several specimens of the hundreds of isopods we have collected and have identified them as *Rocinela bellicepts pugettensis* (Stimpson), a subspecies of *Rocinela bellicepts*. *R. bellicepts* is widespread in northern coastal areas of the North Pacific Ocean, from the Bering Strait southward along the North American coast to California and southward along the Asian coast to Korea (Pavlovskii, 1955). Hatch (1947) feels that most of the specimens in Puget Sound are the subspecies, *pugettensis*. He assigns subspecific rank on the basis of the number of spines on the propodite of the prehensile legs—six spines on the propodite of *pugettensis*, whereas *bellicepts* has three to four. A zone of mixed populations of *R. bellicepts bellicepts* and *R. bellicepts pugettensis* exists near the entrance to Hood Canal, just inside the Strait of Juan de Fuca. All of the specimens we examined from our collections at the Manchester station (which is on the western side of central Puget Sound) had six spines on the propodite of the prehensile legs.

Rocinela sp. belongs to the family Aegidae. Members of this family are widely recorded ectoparasites of fishes. The first three pairs of walking legs are prehensile and are used effectively for attachment.

We originally felt that the attacks on our salmon fry and fingerlings by *R. belliceus pugettensis* might have been the result of confinement and would not be likely to occur with such frequency in open waters. However, on at least three occasions, we have observed wild chum salmon (*O. keta*) and pink salmon fry and fingerlings attacked by *R. belliceus pugettensis*. These attacks occurred at night, under a light, that probably attracted larger numbers of fish and isopods than would normally occur in open waters. In each instance, an isopod attached itself at a point just posterior to the dorsal fin and on, or slightly below, the lateral line. Afflicted fish could not maintain a normal swimming position in a school and darted about in erratic patterns. On one occasion, an afflicted fish was seen to leave the school and disappear. Even if a wound inflicted by an isopod were not fatal, it is possible that the erratic behavior of a fish trying to dislodge the parasite might attract predators.

On another occasion, in one of our large floating pens, we found a juvenile coho salmon with *R. belliceus pugettensis* attached anterior to the dorsal fin and just above the lateral line (Figure 1). This pen was not near our night light and there was a large amount of free space compared with our tanks or cages.

Also, in a cage that had a submerged light to attract plankton, we found *R. belliceus pugettensis* on an immature coho salmon weighing about 200 g. The fish appeared to be in some distress, but perhaps because of its size, the wound was not fatal.

Arai (1969) reported on 68 taxa of parasites recovered from 61 species of fish in British Columbia, but these did not include *R. belliceus belliceus*. Arai's collections were made by seine and trawl; we found that *R. belliceus pugettensis* will release its grasp if the host is forced into

a restrictive net. Hatch (1947) states that *R. belliceus belliceus* is found in 9 to 1250 m of water and that it is an ectoparasite of cod, sculpin, halibut, skate, and other bottom fish. At Manchester, where our observations were made, the water depth is 9 to 13 m. The abundance of *R. belliceus pugettensis* varies between seasons, with the greatest abundance from April through August.

Although we have no way to judge the extent of natural predation of Pacific salmon by *R. belliceus belliceus* or *R. belliceus pugettensis* in the wild, we think that, especially under the confined conditions of pen rearing, the fry and juveniles of Pacific salmon should be included as possible prey of *R. belliceus pugettensis* and probably *R. belliceus belliceus*.

The present instance clearly points up the possible misapplication of the term "parasitism" in certain cases of specialized predation. The isopod *R. belliceus pugettensis* is not a permanent symbiont of a fish and is thus not properly referred to as a parasite.

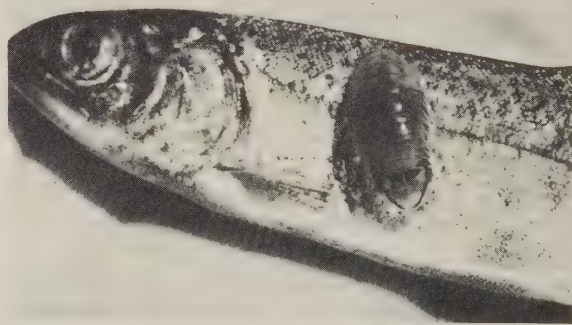


FIGURE 1.—*Rocinela belliceus pugettensis* on a juvenile coho salmon in one of the large growing pens. This specimen was anterior to the dorsal fin, whereas most of the others were attached in a posterior position. Note the blood-filled gut of the isopod.

The authors would like to thank Dr. Paul Ilg of the Department of Zoology, University of Washington, for his suggestions on terminology and Dr. Thomas E. Bowman of the National Museum of Natural History, Washington, D.C., for verifying our identification of the isopod.

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Fishery Bulletin, Vol. 69, No. 2

KELLY, JOHN A., JR., CHARLES M. FUSS, JR., AND JOHN R. HALL, "The transplanting and survival of turtle grass, *Thalassia testudinum*, in Boca Ciega Bay, Florida," p. 273-280.

- 1) Page 274, column 1, 13th line from bottom should read:

A rectangular area 8.2 by 2.1 m (27 by 7 ft)

- 2) Page 278, Table 2, spanner head "Surviving plants" should be separated from "Mortality" by a vertical rule that is continuous with the vertical rule between the subspanner heads, "Successful" and "May."

- 3) Page 278, Table 2, "Total, Construction rod" under "Sept." should read:

0

CALDWELL, DAVID K., MELBA C. CALDWELL, WARREN F. RATHJEN, AND JOHN R. SULLIVAN, "Cetaceans from the Lesser Antillean Island of St. Vincent," p. 303-312.

- 1) Page 309, Table 1, footnote 2 does not apply to the numbers on the December line. "December" under "Average" should read:

4.2

LEONG, RODERICK, "Induced spawning of the northern anchovy, *Engraulis mordax* Girard," p. 357-360.

- 1) Page 357, column 2, last two lines are transposed; text should read:

10.0 mg of salmon (*Oncorhynchus tshawytscha*)
pituitary^a prepared essentially by the method of

PRITCHARD, AUSTIN W., JOHN R. HUNTER, AND REUBEN LASKER, "The relation between exercise and biochemical changes in red and white muscle and liver in the jack mackerel, *Trachurus symmetricus*," p. 379-386.

- 1) Page 379, abstract, portions of lines seven and eight are transposed; text should read:

ming. High lipid content was characteristic of red muscle and was insignificant in white muscle. Lipid use was slow and not correlated with fatigue. A decline in lipid concentration after exercise

